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Vol. 43 March, 1960 Number 3

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of the
AMERICAN DAIRY SCIENCE ASSOCIATION
at Scenic
UTAH STATE UNIVERSITY, LOGAN
JUNE 19–22, 1960

THE FOLLOWING ISSUES OF THIS JOURNAL ARE NEEDED TO TAKE CARE OF BACK ORDERS

1919	January, March, May, July, September, November	1935	December
1920	January, March, May, July, September, November	1936	August, September
1921	January, March, May, July, September, November	1938	February, July, November, December
1922	January, May	1939	December
1923	January, March, May, July, September, November	1943	January, April
1924	January, March, May, July, September, November	1946	January, March, April
1926	January, March, May, July, September, November	1947	January, February, March, April, May, December
1928	January, November	1952	June
1930	May	1953	January, February, September
1931	January, March, May, November	1955	January, April
1932	July, September, November	1956	August
1934	October	1957	February, June, October
		1958	March, June
		1959	January

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for informatiin concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

Advertising

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Nonmember subscriptions are $15.00 per volume in the United States and Canada; $15.50 in all other countries, net and postpaid. New subscriptions and renewals begin with the first issue of the current volume. Renewals should be sent in by December 1 of the current year for the following calendar year to avoid a break in the series. Subscriptions should be sent to the Journal of Dairy Science in care of The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

Changes of mailing addresses, inquiries about copies lost in the mail, requests for back copies and all other matters pertaining to the mailing list for members of the American Dairy Science Association should be sent to Mr. Judkins at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

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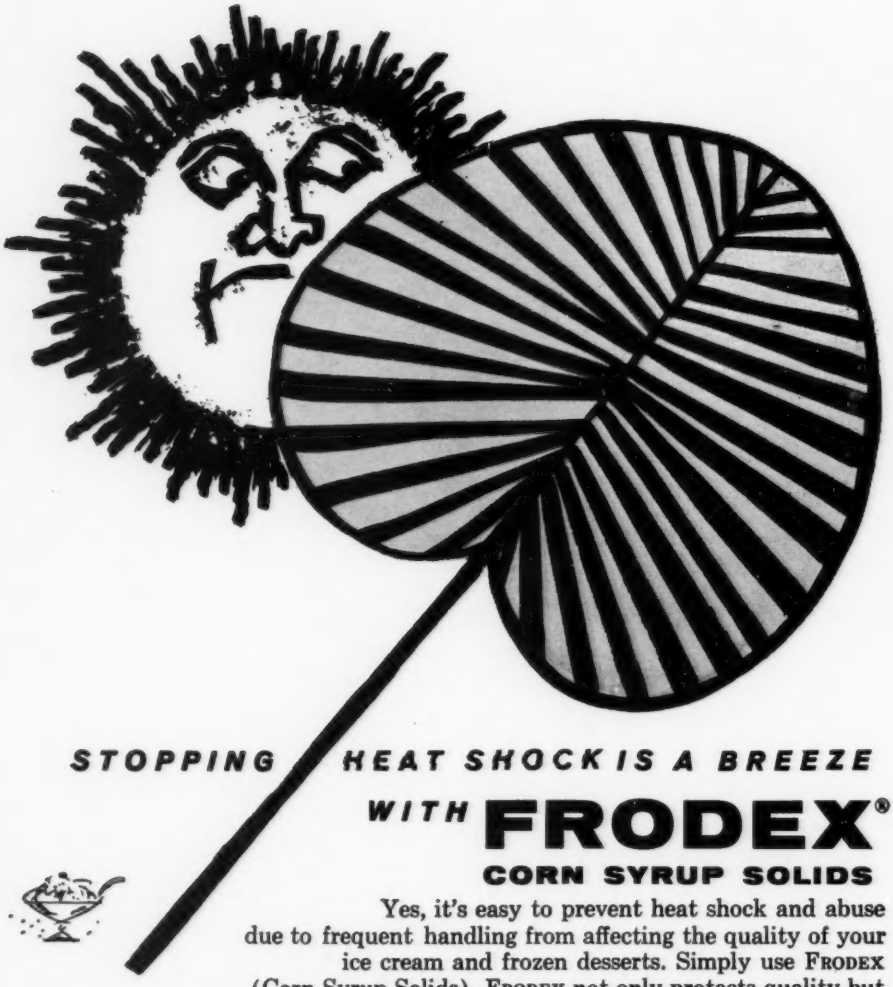

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PEOPLE AND EVENTS

J. Carroll Bateman Appointed General Manager I.I.I.

J. CARROLL BATEMAN was recently appointed general manager of the new Insurance Information Institute (I.I.I.).

Mr. Bateman, 42, has been in the public relations field for 18 yr. He assumed his new position with the I.I.I. on February 1 in New York City. He was the unanimous choice from over 50 candidates.

J. C. Bateman

The Insurance Information Institute for the first time in the industry's history combines all the public relations facilities and operations of eight nationally established capital stock organizations representing 300 fire and casualty insurance companies.

Charter members of the I.I.I. include the Association of Casualty and Surety Companies, National Board of Fire Underwriters, National Bureau of Casualty Underwriters, Surety Association of America, National Automobile Underwriters Association, Inter-Regional Insurance Conference, Inland Marine Underwriters Association, and Inland Marine Insurance Bureau.

Born July 13, 1917, at Norwood, Pa., Mr. Bateman attended Baltimore (Maryland) schools, was graduated from Johns Hopkins University, and attended the Graduate School at New York University.

A former instructor and lecturer in public relations at Johns Hopkins University and the Bernard Baruch School of Business Administration, Mr. Bateman also has been a guest lecturer at Ohio University, Loyola College, and the Universities of Baltimore and Maryland.

Active in community and professional groups, Mr. Bateman is a member of and public relations advisor for the Board of Trustees of Family and Child Services in Washington, a member of the Official Board of the Potomac Community Church, the Public Relations Advisory Committee of the U. S. People-to-People Program, and the Editorial Board of PR Quarterly, published in Washington.

He is vice-president of the Washington Chapter of the Public Relations Society of America, a member of the American Public Relations Association, American Society of Association Executives, the National Press Club, Army and Navy Club of Washington, the Johns Hopkins Club of Baltimore, Railroad-Machinery Club of New York, and The Newcomen Society in North America.

During World War II, Mr. Bateman served for nearly 4 yr. with the infantry and rose from private to captain. He was a member of the Army Reserve until 1956. He is married and has one daughter and a son.

P. R. Ellsworth Named Associate Director of Milk Industry Foundation

P. R. ELLSWORTH, formerly assistant to the Executive Director of the Milk Industry Foundation, has been promoted to Associate Director, it was recently announced.

A graduate of Ohio State University with the class of 1942, Mr. Ellsworth served in the United States Army for 4 yr., rising to the rank of Major in the Artillery. After returning from World War II, he managed a farm for a dairy company at Cleveland, Ohio, during 1947, and subsequently became Extension Specialist in Dairy Technology for the Ohio State University at Columbus. For 5 yr. of this period, he also served as secretary-treasurer of the American Dairy Science Association.

P. R. Ellsworth

In his position at the Foundation for the last 5½ yr., Mr. Ellsworth at one time or another has been in producer relations program, and the development of the Foundation's plant and fleet accident program, and also has served as assistant convention manager.

New Dairy Scholarships Established at Minnesota

Two new scholarships have been established by the Minnesota Dairy Industry committee for undergraduate work in dairy production at the University of Minnesota.

The two grants of $300 each were founded in honor of W. S. Moscrip of St. Paul, internationally recognized dairy cattle and dairy industry authority who was killed in an auto accident November 1, 1959.

Moscrip, who originated the true type standards in dairy cattle breeding and judging, was a founder and past-president of the Minnesota Dairy Industry committee, from which grew the American Dairy Association.

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Recipients of the scholarships will be chosen according to academic aptitude and high school achievement, vocational promise and demonstrated work in dairy production, personal attributes of citizenship and character, and potential for leadership.

Interested high school seniors may apply either to the Department of Dairy Husbandry or to the Institute of Agriculture office on the University's St. Paul campus. Final selection will be made by a regular scholarship committee of the University. The first two scholarships will be available in the fall of 1960.

NOTES FROM THE SECRETARY'S DESK

Procrastination

At the risk of sounding rather trite, I am going to write something about procrastination. I feel qualified to write on this subject because in all of my business life up to this very moment I have probably been as busy as the average man, to say the least, and have always tried to take care of my commitments as promptly as possible. There can be no question but that procrastination is one of the principal deterrents in conducting business in a prompt and efficient manner.

H. F. Judkins

Why does one procrastinate on various matters, notably replying to letters, paying bills on time, etc.? Is it because one is *really* so busy that there just aren't enough hours in the day to take care of all of the demands upon him? Or, is it because of a failure to organize his work, due to carelessness or poor planning resulting in not putting first things first? Or, does he believe in the statement sometimes made, that given time enough any letter will answer itself? Or, is it because an inquiry seems much less important to the one receiving the inquiry than it is to the inquirer? Or, is it because of lack of thought on the effect that his procrastination may have on the persons with whom he is doing business?

I will mention a few recent instances of procrastination that have affected the work of your Secretary or the affairs of the Association.

Take the matter of Awards nominations that come in with dues payments. The nomination blank states in capital letters that nominations must reach the Secretary by December 15, yet nominations have been received even after January first.

In the matter of payment of dues, the dues notice states in capital letters that remittance

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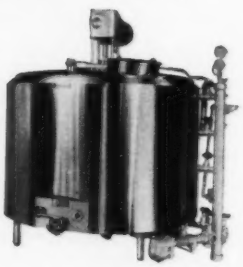

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should be made by January first, and it has been explained time and again that those who have not paid their dues by January first must be suspended, which means that they will receive no more Journals until and unless they are reinstated. On January 1, 1960, there were 646, 1959 members who had not renewed. This meant a vast amount of clerical work in stamping "Suspend" cards and sending them to the printer who, in turn, has to remove his address plates from his active file. The cards are then returned to the Secretary and as dues are paid they must be stamped "Reinstated" and again sent to the printer who must change his address plates from the suspend file back into the active file, the cards then being returned to the Secretary for his records. A mistake is occasionally made—is it any wonder? It also means sending out another dues notice to those who have not renewed, as well as a list of their names to each State Chairman of the Membership Committee. I am happy to say that as a result of this work, many renewals are being received each day and it is hoped that the Membership Committee's goal for members for 1960 may be reached soon.

A few always wake up to the fact of the January first deadline on or about December 30 or 31 and rush their dues in by air-mail. One came in air-mail, special delivery—postage 37¢. At that, if procrastination cost only 37¢ each time it wouldn't be so bad.

Take the matter of Affiliate memberships. On September 15 the Secretary sent a letter to Department Heads concerning the eligibility of 1959 Affiliates for the year 1960, and a request for the names of those who would be eligible for full membership for 1960. Again, the deadline for the receipt of this information was December 15. New Affiliate dues received by that date entitle the member to 15 issues of the Journal instead of 12. Some replies to the inquiry of September 15 have not been received as of January 22. One was received on December 22. As of December 15, 157 new Affiliate memberships had been received, whereas on January 22, 194 had been received, meaning that as a result of somebody's procrastination, 37 new Affiliate members will get only 12 Journals instead of 15 and there will be more before the "season" is over.

In the matter of correspondence, I would hate to say the number of times more than one letter is required to get the information desired and sometimes even a long-distance telephone call has to be resorted to.

It is refreshing that once in a while a member will realize what his procrastination has meant and will write me a note, as for example two received recently, reading as follows:

> It is most regretted that I neglected the renewal prior to December 1. Your generous forgiveness is highly appreciated.

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and the other says:

> I am sorry to be so late with my membership dues, but I mislaid this notice and did not find it until today. I know that this is an inconvenience for you and extra work.

I couldn't say it better!

Carelessness

As a corollary to my message on "Procrastination," I feel constrained to write another on the subject of carelessness. No pious statements concerning carelessness are necessary, as everybody knows what it is. I simply want to give some examples that appear each year in connection with the collection of Awards nominations and membership dues.

The Awards nomination blank clearly defines the different awards, and provides a place for the name of the nominee and his state and a place for the signature of the nominator and his state. Here are some of the problems:

1. Names are frequently not typed or printed and in some instance it is impossible to read them. In one or two cases nominations were made but the blank was unsigned, so it had to be thrown out.

2. Sometimes the state is omitted. This makes it very difficult for us to check the person in our card file of 2,600 members, which is set up by states and alphabetically within the state. Due to labor involved, we have not deemed it wise to carry a second alphabetical membership file.

3. This year's nomination blank plainly stated that the Teaching Award was to go to a Production man and yet out of 23 nominations made, 9 were for Manufacturing men who, of course, were ineligible.

4. We never have had anywhere near complete information as to the type of business and type of position held by members and whether their major interest was in Production, Manufacturing, or Extension. On this year's dues notice there was space to write in:

Name of present employer .....
Type of business .....
Type of your position .....

At least 200 failed to fill in these spaces when paying their dues and this has meant writing letters to these members in an attempt to get this information. In other instances what was written could not be deciphered. In a few cases there was failure to check either Production, Manufacturing, or Extension Section.

There are a number of instances when it is important to know what the interest of the member is and it can have an important bearing on the content of our Journal and the arrangement of our Annual Meeting program. We didn't just ask for this information to make ourselves more work!

5. In a few instances, dues notices were returned properly executed, but no check was included in the envelope, which meant correspondence.

6. In a considerable number of instances, dues are paid by check, sometimes a company check, and the dues notice is not returned with the check. Sometimes correspondence is necessary to run these down to be sure that the payment is credited to the right party. The dues notice always states in capital letters, "Please Return this Bill." Of course, in these instances we do not get the information concerning the interest of the member and, again, more correspondence.

7. What is said in No. 6 is not confined to dues notices. Frequently, invoices for reprints or back copies are paid by check without any indication whatsoever as to the purpose for which the check is intended. This results in more correspondence.

Let us all remember that carelessness can not only be very aggravating but costly to the Association in clerical time and postage.

—H. F. JUDKINS

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News From Illinois

Fresh and Sterile Concentrated Milk Discussed at Illinois, February 2, 1960

As a part of the Agricultural Industries Forum, a half day was devoted to a discussion and demonstration of fresh and sterile concentrated milk.. Those particiipating in this program were CHARLES STUBE, of Rochester, Minnesota; C. IVERSEN, of Iowa State University, Ames; A. M. SWANSEN, University of Wisconsin, Madison; A. P. STEWART, Corning, Iowa; ARTHUR ROBINSON, Arlington, Virginia, and E. O. HERREID, University of Illinois, Urbana.

Trade barriers in the sale of fresh concentrated milk were discussed by A. L. MCWILLIAMS, Chicago, Illinois, and a program to eliminate such trade barriers was discussed by SYDNEY BERDE, Attorney General's Office, St. Paul, Minnesota. Actual and potential markets for fresh and sterile concentrated milk were presented by R. W. BARTLETT of the University of Illinois.

A. P. Stewart emphasized production and sale of sterile concentrated milk, and W. B. COOPER discussed the production and sale of sterile milk. The promotional and selling aspects of sterile concentrated milk were elaborated upon in detail by L. R. BRUCE of the Compton Advertising Company of New York City.

The demonstration of fresh and concentrated milks, which was held in the Dairy Manufactures Building, was attended by 220 people from different sections of the United States and from four provinces in Canada.

A New Publication for Industry

The Beck Vanilla Products Company of East St. Louis, Missouri, is publishing a new pamphlet called Tracings. The Editor is P. H. TRACY, emeritus Professor of Dairy Technology, University of Illinois, Urbana. His present address is P. O. Box 153, DeLand, Florida.

HOWARD BECK, president, is quoted in Tracings, "There has never been a time in the history of our country when the complexion of business was changing as rapidly as it is today. If a company is to survive it must have good leadership. Management must produce efficiently, and products must be of high quality. Naturally we want our customers to remain in business and operate at a profit. As a business aid we have recently established a technical service. It is our goal to contribute what help we can to make your operations successful, and we would be glad to have you contact us with your management problems."

Dairy Technology Conference

Law Building
University of Illinois, Urbana
April 12-13, 1960

April 12, P.M.—GENERAL SESSION

1:00—Welcome. Dean L. B. Howard
1:40—Planning Ahead. E. M. Norton, Natural Milk Producers Federation, Washington, D.C.
3:00—Insecticides and Antibiotic Residues. R. P. Link, University of Illinois
3:45—Consumers' Opinions of Additives and Insecticides. Marian Ingersoll, Champaign, Illinois
7:30—Products Judging and Demonstrating. Judging Market Milk. Everett Byers, E. O. Herreid, Ashley Wilson
Judging Ice Cream. W. C. Corbett, O. E. Ross, J. Tobias
Judging Fermented Products. J. H. Petersen, S. L. Tuckey, H. K. Wilson
General discussion of all products. R. E. Lloyd, Moderator.

April 13, A.M.—PROCUREMENT

8:45—Educational Approach to Problems of Milk Procurement. L. R. Davenport, Department of Health, and L. D. Oranger, Department of Agriculture, Springfield, Illinois
9:30—Do Quality Tests Tell Us What We Need to Know? H. E. Calbert, University of Wisconsin, Madison
10:30—Testing for Antibiotics. L. D. Witter, University of Illinois
11:15—Problems Facing Producers in 1960. J. G. Cash, University of Illinois

April 13, A.M.—PROCESSING

8:45—Processing Efficiency. V. E. Meyers, Millville, Pennsylvania
9:30—Automatic Valves. Arthur Dalton, Chicago, Illinois
10:30—Effects of High Temperatures on Properties of Fluid Milk Products. Robert Jenness, University of Minnesota, St. Paul
11:15—Control of Psychrophils in Cottage Cheese. S. L. Tuckey, University of Illinois

April 13, A.M.—MERCHANDISING

8:45—Give Consumers What They Want. R. C. Lakamp, Cincinnati, Ohio
9:30—Changing Patterns in Dairy Products Consumption. Mardy Myers, Washington, D. C.
10:30—Private Labels. Ray Higgins, Chicago, Illinois
11:15—Packaging Dairy Products. L. J. Hayrust, Chicago, Illinois

12:15—Lunch.

Protecting the Health of Executives. F. R. Steggerda, University of Illinois

Southern Illinois University News

Fifth Annual Dairy Day was held at Southern Illinois University, Carbondale, March 2, from 10 A.M. to 3 P.M.

Talks were given on breeding of dairy cattle, pasture research, and cow pools. The following university-associated men participated: ROBERT TOUCHBERRY and E. E. ORMISTON, University of Illinois; ROBERT LUSH, University of Tennessee; and H. H. OLSON and ALEX REED, Southern Illinois University.

News from Ohio State

W. L. SLATTER and family have returned to Ohio from India for a two-month leave of absence before beginning another 2-yr. period as a member of the Ohio State University team under the Technical Cooperation Mission to India. Dr. Slatter reports that considerable progress has been made during the past 2 yr. on the Indian Dairy Research Institute at Karnal, Punjab. Within the next 2 yr. they hope to complete the dairy manufacturing department and the dairy plant. The Slatters will return to India, February 2, via San Francisco, Hawaii, and Hong Kong.

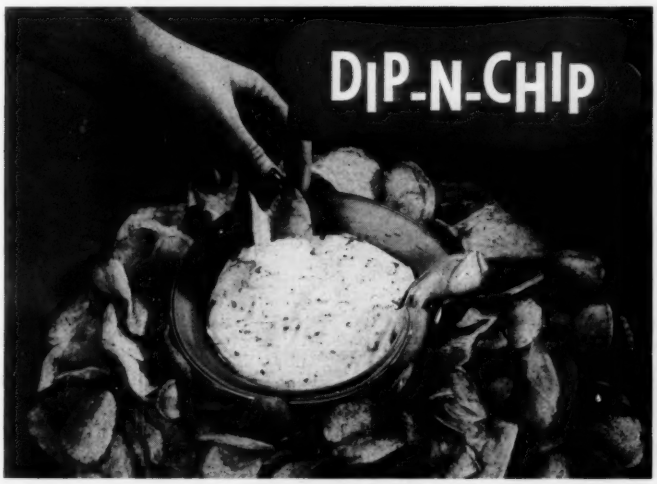
The following staff changes have been announced:

M. L. PEEPLES, Instructor in Dairy Technology, has resigned effective January 31 to return to Texas Institute of Technology, Lubbock, Texas, where he has been on a 2-yr. leave of absence.

J. J. BETSCHER, M.S. 1958, The Ohio State University, has been appointed Instructor in Dairy Technology effective January 1. Mr. Betscher's major area will be Dairy Engineering.

C. E. COBB, B.S. 1947, The Ohio State University, has been appointed Instructor in Dairy Technology and Assistant Dairy Plant Manager, effective January 1. Mr. Cobb has for the past 10 yr. been associated with the Hagan Ice Cream Company, Uniontown, Pennsylvania, as Production and Quality Control Supervisor.

The Ohio Dairy Products Association held its 43rd Annual Convention, January 25-27, in Columbus. The theme of the meeting was Facing the Issues—in the '60's. The subjects discussed pertained to dairy problems of national and state-wide concern such as legislation, taxation, consumer trends, sales and selling, labor and management, and school milk programs. Speakers included ROBERT NORTH, IAICM, Washington, D. C.; ED CLARK, Joe Lowe Corporation, New York; PAUL POTTER,



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Chicago; COURTNEY JOHNSON, Beatrice Foods Company, Chicago; HAROLD GREEK, Columbus, and WADE BASH, Columbus.

Officers of the Ohio Dairy Products Association for 1960 include: President: S. L. JOHNSONBAUGH, Defiance Milk Products, Defiance; vice-president: P. J. BETSCHER, French-Bauer, Inc., Cincinnati; treasurer: J. L. BURNS, Beatrice Foods Company, Columbus. Milk Distributors. president: E. C. GREINER, Greiner Dairy Company, Zanesville; vice-president: O. A. DEAN, II, O. A. Dean Dairy Company, Cleveland; past-president: ED MOSER, Med-O-Pure Dairy, Washington C. H.

Milk Products Manufacturers. president: JOHN MARABELLA, Carnation Company, Coshocton; vice-president: R. J. GILBERT, Pet Milk Company, Bryan; past-president: RICHARD BUEHLER, M R Dietetic Labs., Inc., Columbus. Ice Cream Manufacturers. president: R. L. BAYNTON, Harmony Farms, Columbus; vice-president: O. G. HOFFMAN, Royal Crest, Dayton; past-president: ROGER UMBS, San-A-Pure Dairy, Findlay. Butter Manufacturers. president: EARL GRIESSER, Napoleon Creamery Co., Napoleon; vice-president: J. A. HYEST, Cloverland Dairy, Flushing; past-president: HAROLD HAMILTON, Eldorado Creamery, Eldorado. ROBERT WEBNER, Cottage Creamery Company, Orrville, will serve one more year on the Board as past-president.

Department of Dairy Science Created at Kentucky

A Department of Dairy Science has been created at the University of Kentucky by recent action of the University's Board of Trustees. D. M. SEATH will head the new department.

The action was taken to recognize the growing importance of dairying in Kentucky's agriculture, according to F. J. WELCH, Dean of the University's College of Agriculture and Home Economics. He also stated that the change was in keeping with the administrative organization of many land grant institutions throughout the country.

For many years dairy science at the University of Kentucky has been administered as a section of the Animal Industry Group. The action of the Board of Trustees divided the Group into three departments—Dairy, Animal Husbandry, and Poultry.

Dairying is becoming one of the major agricultural enterprises in Kentucky. It has a wide reputation as a tobacco-producing state. Few people realize that in 1958, as in several years recently, the dairy enterprise contributed over $100,000,000 to the farm income. This is more than half of the income from tobacco. The state ranks 13th in total milk production, 3rd in Cheddar Cheese production, and 4th in evaporated milk output. Furthermore, with changing economic conditions, changes are being made in outlets for farmers' milk. Examples of these changes are the reduction in numbers of cream-buying stations and construction of new fluid milk plants, milk drying plants, and cheese factories in the state.

Work in the new department will continue as in the past in the fields of dairy production and dairy manufacturing. Extension service in both fields is expanding. Dr. Seath, in conjunction with his duties as department head, will supervise the research, teaching, and extension work in the dairy animal field. T. R. FREEMAN, professor in charge of dairy manufacturing, will supervise work in that area.

Research work in the new department includes work in pasture nutrition studies, calf nutrition work, rumen physiology studies, studies in reproduction of dairy cattle, cattle appetite studies, genetic studies in dairy cattle, and bacteriological and chemical problems related to market milk, cheese, butter, and frozen cultures.

Massachusetts News

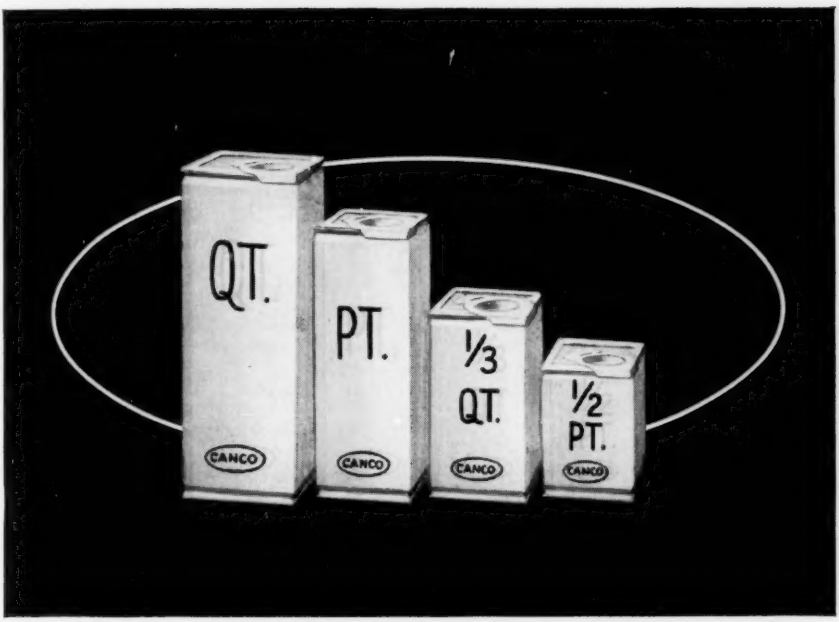
Ice Cream Short Course

Thirteen students attended the annual Ice Cream Short Course at the University of Massachusetts, from January 11 to 22, 1960. Enrolled in the course were seven in the manufacturing phase of ice cream, and six in the supply field, from six different states—Massachusetts, Maine, New Hampshire, Rhode Island, Connecticut, and New York. The short course consisted of lectures, laboratories, and demonstrations covering various phases of ice cream production.

Those attending the course were G. D. COLWELL, The Hubinger Co., Norwich, Conn.; R. H. DEREDON, DeRedon Food Products Corp., Niantic, Conn.; DONALD GREEN, H. A. Johnson Co., Fairhaven, Mass.; M. R. GREEN, Crystal Spring Dairy Bar, Belchertown, Mass.; C. F. HUNT, Pittsfield Milk Exchange, Pittsfield, Mass.; C. H. LAWRENCE, JR., The Hubinger Co., Holbrook, Mass.; JACK LUSK, A. E. Staley Mfg. Co., Providence, R. I.; LAWRENCE MACKENZIE, MacKenzie Dairy, Keene, N. H.; R. C. MACKENZIE, Select Ice Cream Co., Quiney, Mass.; R. W. MILLER, JR., Rivers Ice Cream, Chicopee Falls, Mass.; P. C. MOREAU, Corn Products Sales Co., Amityville, N. Y.; P. M. PAYSON, Rivers Ice Cream, Chicopee Falls, Mass.; and FRANK THURSTON, Grant's Dairy, Bangor, Maine.

At the annual Ice Cream Forum Dinner on Thursday evening, January 21, the following awards were presented to the outstanding students in the Short Course:

The D. H. Nelson Memorial Award, consisting of a $25 Savings Bond, donated by The Hubinger Company, was presented to P. M. PAYSON, Rivers Ice Cream, Chicopee Falls, Mass., for being the outstanding student in the Course.



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★ ECONOMICAL! Saves time, reduces labor and equipment costs.
★ Approved by American Dairy Science Association June, 1959 and adopted as first action for fat testing of raw milk by Association of Official Agricultural Chemists October, 1959, subject to State regulations. Also approved for DHIA testing.

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P. C. MOREAU, Corn Products Sales Co., Amityville, N. Y., received a pen and pencil set, donated by the H. A. Johnson Company, for being the best student involving sales personnel.

For having the best knowledge of ice cream defects, DONALD GREEN of the H. A. Johnson Company received the book Judging Dairy Products, donated by JOSEPH MCDONALD of Providence, R. I.

The Short Course was under the direction of FRANK E. POTTER, Assistant Professor of Dairy Technology.

Ice Cream Forum

One hundred and ten ice cream manufacturers and suppliers attended the Ice Cream Forum on the University of Massachusetts campus, January 21-22, 1960. In addition to the very informative presentations by the speakers, manufacturers submitted 22 samples of vanilla ice cream and 12 samples of coffee ice cream for the annual Ice Cream Clinic.

CHESTER CHENEY of Hendries Ice Cream discussed The Vacuum Treatment of Ice Cream Mix in relation to his experiences during the past year with this processing procedure. The ever-timely subject of Selecting Dairy Products for the Mix was presented by MEYER GLICKSTEIN of Sealtest Foods.

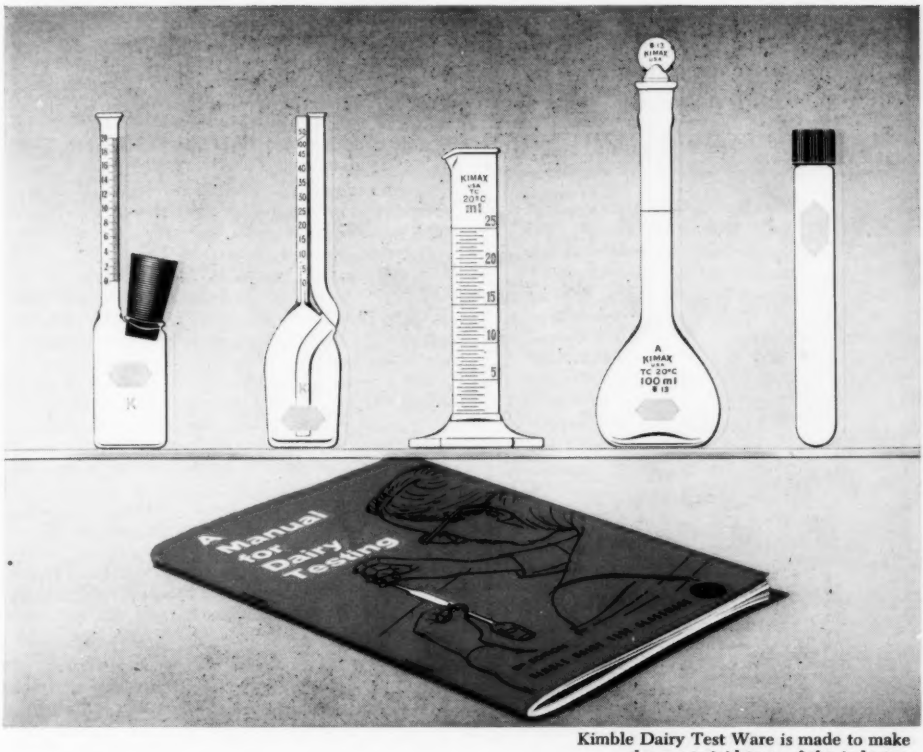
A very informative and interesting talk by REX PAXTON of Sutherland Paper Company, entitled Double Your Advertising Dollars with Packaging, was widely accepted by the Forum audience.

In the afternoon session, the team consisting of JOHN SULLIVAN of the Small Business Administration; FRANCIS BRENNAN, Massachusetts Business Development Corporation; and REGINALD COLE, National Bank of Plymouth County, discussed the subject of credit for the small business. This was followed by group discussions on production problems, batch freezer operation, merchandising, and credit.

The Friday morning session, following the Ice Cream Clinic, consisted of talks on Flavoring Coffee Ice Cream by WILLIS STEINITZ of American Food Laboratories, and Quality Control in Ice Cream Manufacture by C. W. ENGLAND of Washington, D. C. CHARLES STICKNEY of Deering Ice Cream concluded the program with an excellent presentation on timely business tips for the small ice cream manufacturer.

Florida News

JACK GAY, a senior student in the Department of Dairy Seience at the University of Florida, was the recipient of the 1959-60 Virginia Dare Award. The presentation was made by LEON E. MULL for the Virginia Dare Extract Company of Brooklyn, New York, at Honors Day ceremonies held recently by the



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Department of Dairy Science in recognition of outstanding students.

Gay, whose home is in Clearwater, Florida, plans to operate his own dairy enterprise in Clearwater following graduation in January, 1960.

Pennsylvania State University Research Team Honored For Dairy Project

A three-man research team from the Dairy Breeding Research Center at the Pennsylvania State University was honored recently for outstanding research.

J. O. ALMQUIST, R. J. FLIPSE, and W. T. O'DELL received a $300 cash award and certificate for development of a milk-glycerol diluter for use with bull semen. The award was made by the Glycerine Producers' Association.

The Penn State studies show that addition of glycerine (glycerol) to a heated milk diluter prolongs the fertile life of sperm cells at least four days after collection. Liquid semen preserved in a milk-glycerol diluter is cheaper and easier to use than frozen semen.

The work of the team reduces the time needed for collection and distribution of semen, since quality is well preserved with the diluter. Dairymen also have a wider choice of sires due to greater availability of semen. Artificial breeding associations in Pennsylvania claim excellent results with milk-glycerol diluted semen.

Nebraska

I. L. HATHAWAY, Professor of Dairy Husbandry at the University of Nebraska, passed away January 6, 1960, after a protracted illness. He was born December 28, 1899, at Redfield, Iowa.

Professor Hathaway received the B. Sc. degree from the Kansas State University in 1925 and the M. Sc. degree in 1926. He came to the University of Nebraska in 1926 and was advanced to a full professorship in 1955. At Nebraska he taught a few classes in small animal laboratory techniques, but his main efforts were devoted to research. He was active in studies of growth in dairy cattle, the feeding of dairy calves, and nutritional studies, particularly those having to do with the vitamins. In later years, he had been making extended research in the vitamin content of various cheeses and was considered an authority in that field.

He was the author or co-author of many scientific papers published in scientific journals and in the agricultural experiment station bulletins.

In the University of Nebraska he was a member of the Senate Committee on Committees and of the Committee on Student Affairs. For many years he acted as advisor of the Agricultural Executive Board. He had served as a deacon in the First Plymouth Congregational Church, and was currently serving as a member of the Board of Directors of the Lincoln Rotary Club.

In University circles Professor Hathaway was known for his careful scientific work and his faithful carrying out of every assignment.

Mrs. Hathaway, a daughter, Mrs. Harry Spencer, Jr., and two grandchildren of Long Beach, California, and one sister, Mrs. L. Strause of Sheffield, Iowa, survive.

Georgia

A Cottage Cheese clinic and demonstration was held in the Dairy Science Building, February 9-10, 1960. It was under the supervision of O. D. Sousek, National Pectin Co., and members of the local staff.

The Management Development Conference for dairy plant executives and supervisors will be held at the Georgia Center for Continuing Education at the University, April 24-27, 1960, under the sponsorship of the Dairy Department. University specialists in their respective fields will discuss functions and responsibilities of management, policy formation, communications, personnel and administrative factors, personnel selection and evaluation, labor relations, budget and fiscal control, credit control, dairy plant training, and other subjects.

An educational tour through the Midwest for junior and senior students in dairy production and in dairy manufactures is being planned for the interval between the winter and spring quarters. Transportation is sponsored by the Dairy Science Club from profits earned in dairy enterprises. It is hoped that such tours may be conducted biennially.

The Dairy Science Club banquet was held on January 29 in the Georgia Center for Continuing Education. D. W. Brooks, Manager of the Cotton Producers' Association, was the principal speaker. A feature of the program was the presentation of citations and brochures of letters from the University administration and student recipients of scholarships to scholarship donors.

C. M. Clifton joined the resident teaching and experiment station staff on January 1, 1960, as Assistant Professor of Dairying. He is a native of Kentucky and received his B.S. degree from Eastern State College of Kentucky, M.S. from the University of Kentucky, and Ph.D. from Ohio State University. He has held positions in the Greenup County, Kentucky, High School, Eastern State College, and the Dairy Departments of the University of Minnesota and the University of Kentucky. He has served as a gunnery instructor in the U. S. Army Air Corps. He has published many research articles on dairy cattle breeding.

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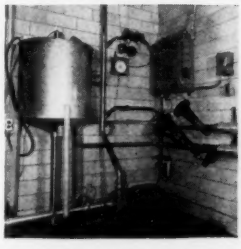

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BELOIT WISCONSIN

MRS. FREDERICK W. BENNETT passed away on January 2, 1960, at the age of 57, after an illness of about three weeks. She was a native of Atlanta and an honor graduate of the State Normal School which later became a part of the University of Georgia. She was an energetic leader in the Athens First Baptist Church in which she held most of the positions open to women, and in regional and state women's organizations of the denomination. She was also a member of the University Woman's Club and other civic groups and an ardent worker in the W.C.T.U. She very actively assisted her husband as Faculty Advisor of the Baptist Student Union at the University. She took much interest in the many annual meetings of the American Dairy Science Association which she attended. The survivors include her husband, who is a member of the dairy staff; a daughter, MRS. ROBERT W. JETER, Shreveport, La.; a son, R. L. BENNETT, Atlanta; a sister, MRS. C. H. HORNE, College Park, Georgia; and two grandchildren.

A. O. Smith Corporation Establishes New Position

P. A. LINDER has been appointed manager of engineering for the A. O. Smith Corporation. L. M. OSBORNE, general manager, announces that in his new position Mr. Linder will be responsible for all engineering developments and design of both agricultural and industrial products.

P. A. Linder

Linder is one of the first agricultural engineers to specialize in mechanized feedlot layouts. A mechanical feeding system which he designed and built on the family farm in 1954 was featured in a motion picture produced by Iowa State College. This system was one of the first completely mechanized feeding systems ever designed and built, and included metering devices so that accurately blended rations were fed.

Mr. Linder graduated from Iowa State College at Ames, in 1952, with a B.S. degree in agricultural engineering, and is a member of Alpha Zeta, honorary agricultural fraternity. During the war he served with the U. S. Army combat engineers.

Creamery Package Announces New Changes

An enlarged slate of officers has recently been elected by the Board of Directors of The Creamery Package Mfg. Company, manufacturer of food processing, refrigerating, and materials handling equipment, according to an announcement by the firm's president, E. B. LEHRACK.

Promoted to vice-presidents were: C. Y. MCCOWN, Purchasing; L. M. MOMMSEN, Production; and A. H. WAKEMAN, Engineering and Research; advanced to treasurer is W. A. ANDERSON; G. W. PUTNAM, formerly vice-president of Engineering and Production, is now vice-president of Public Relations. The Company also announced that E. K. DELANA, former treasurer, has been appointed Company Attorney.

D. T. Fitzmaurice Promoted by Cherry-Burrell

Appointment of D. T. FITZMAURICE as Vice-President, Research and Development, was announced recently by H. H. CHERRY, JR., President of Cherry-Burrell Corporation.

D. T. Fitzmaurice

Fitzmaurice joined Cherry-Burrell in 1945. He served as New York branch engineer, senior machinery specialist, division sales manager in the Company's Little Falls, New York plant, and industrial sales manager in the general office in Cedar Rapids. Since 1957 he has been technical director in charge of research and development.

Fitzmaurice received a degree in mechanical engineering at New York City College and was commissioned a Lieutenant Commander in the U. S. Navy during World War II.

C. B. Lane Promoted at Breakstone Foods

Breakstone Foods, Division of National Dairy Products Corp., today announced the appointment of C. B. LANE as Vice-President in charge of Production.

Dr. Lane joined Breakstone's in 1948 as Director of Research and Development at the Walton, N. Y., plant. In 1952, he was appointed Manager of the Walton operations, and in 1959 he was assigned to Breakstone's general offices in New York City.

A graduate of Penn State University, Dr. Lane received his M.S. and Ph.D. degrees from Iowa State University. He was with the Iowa State Experimental Station for 8 yr. doing research work on dairy products, specializing in the chemistry, bacteriology, and enzymology of dairy products with special emphasis on cheese. He is the author and co-author of numerous articles related to dairy research. Dr. Lane is also the co-inventor of three patents presently being used by the dairy industry.

Dr. Lane succeeds HERMAN GOLD, who has

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2	Yes	No
3	Very slight	No
4	Very slight	No
5	Yes	No
6	Yes	No
7	Yes	No
8	Yes	No
9	Yes	No

PENNSAN is the superior bactericide serving the needs of modern sanitization. It removes and prevents milkstone and films, works in even hardest water, does not corrode stainless steel . . . controls bacteriophages without affecting starter cultures. PENNSAN is a unique chemical sanitizer—a new concept to serve *more* sanitizing and cleaning needs.

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Taft Engineering Center Presents Course in Radionuclides in Foods

The course Radionuclides in Foods will be presented April 18-29 at Robert A. Taft Sanitary Engineering Center, PHS, Cincinnati.

It was designed for professional personnel having responsibilities for the surveillance of radioactive materials in milk and food.

A cooperative achievement of the Radiological Health and Milk and Food Training units of the SEC Training Program, the new course first was presented last September, eliciting so much interest that the 200 copies of the first edition of the course manual were exhausted before November 1.

Purposes of the course are to provide technical training in methods for sampling and assay of radioactive contaminants, to discuss procedures for the interpretation of data obtained, and to provide an opportunity for discussion of the problems.

Sessions on radiation fundamentals and instrumentation provide foundation for subsequent discussion of sources of radionuclides in foods, aquatic and terrestrial food chains, milk and dairy product surveillance, sampling procedures, radiochemical procedures, maximum permissible concentrations, and public health significance of radionuclides in foods.

Approximately 40% of the course will be devoted to laboratory work. Because of the specialized equipment and facilities required, the number of trainees who can be accommodated will be limited.

Applications should be addressed to the Chief, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

Summary of Symposium on Germ Plasm Resources in Agriculture: Development and Protection at A.A.A.S. Meeting

The need for additional sources of animal and plant germ plasm was brought out at the symposium on Germ Plasm Resources in Agriculture, presented at the American Association for Advancement of Science meetings in Chicago, December 28-30, 1959. The contrast between methods available for utilizing germ plasm by plant and animal breeders was clearly illustrated in the 26 papers presented. The urgent need for preserving germ plasm of both plant and animal stock was also emphasized.

In the first of the five sessions, J. R. HARLAN, of the USDA and Oklahoma, pointed out that the origins of cultivated crops are diffuse in both time and space. Thus, our crop plants did not originate in any one geographic area. On the other hand, H. H. STONAKER of Colorado showed that the origin of farm animals in the United States is almost exclusively one of importation from Great Britain, Holland, Belgium, France, Spain, and the Channel Islands. R. W. PHILLIPS, now with the Foreign Agricultural Service of USDA, reviewed the many types of cattle, sheep, swine, and poultry which have received little or no attention in the United States. Former Vice-President HENRY A. WALLACE chided the animal geneticists and breeders for not accepting more actively the challenge of discovering good genes outside the United States and infusing them into our present domestic animals. Mr. Wallace praised the plant breeders for their splendid progress in this regard and expressed concern about the need for preserving unique genes in both plants and animals.

In the second session, F. P. CULLINAN and M. G. WEISS of the USDA outlined the continuing need for additional sources of germ plasm in field and horticultural crops. Important among the needs were the development of genetic resistance to domestic and exotic plant diseases, adaptability to climate conditions, and adaptation to mechanization. The existing program of the USDA for plant introduction was mentioned and the need to catalog existing and introduced stocks for genetic composition was emphasized. A. W. NORDSKOG and J. L. LUSH of Iowa discussed the need for additional sources of germ plasm in farm animals. Disease-resistant poultry, improved flavor of broilers, and low-fat ducks and geese were suggested as possible results obtainable by introduction of nondomestic fowl. Lush pointed out that introductions of outside blood can sometimes hasten progress by (1) introducing genes and genetic variation not already present, (2) increasing frequency of rare desirable genes, and (3) introduction of a breed or stock already well suited to some ecological niche in the United States. He pointed out that the benefits of improving existing stocks in the United States have not been exhausted. However, he believed that needless barriers to animal introduction from other countries should be removed. H. A. RODENHISER of the USDA stressed the need for expanded research for new plant germ plasm and supported the proposed National Bank for Farm Animal Germ Plasm, which would provide a facility to acquire, preserve, and distribute frozen semen to federal and state agricultural experiment stations.

In the third session, the importance of diverse germ plasm to the improvement of alfalfa and sweet clover was related in a paper by H. J. GORZ and W. K. SMITH of Nebraska and Wisconsin, respectively. G. W. BURTON of the USDA and Georgia illustrated the many ways in which hybrid vigor had contributed to plant improvement. He showed how commercial hybrids are produced by vegetative means, by apomictic seeds, by hand emascu-

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lation and pollenation, and by exploiting cytoplasmic male sterility self-incompatibility and chance hybridization. L. N. HAZEL of Iowa indicated the extensive use of hybrid vigor in commercial swine and sheep production and reviewed the efforts being made to evaluate its use in other types of livestock. He pointed out that heterosis appears to be most apparent in the early life of the animal. G. E. DICKERSON of Kimber Farms, California, discussed efforts to determine how much progress has been made or could be expected from selection in livestock. He stated that a realistic approach required consideration of the response of all traits, the genetic and environmental interactions, loss from recombinations and evolution of environment. He stressed the need for techniques to measure environmental changes.

N. D. BAYLEY reported for the USDA on the progress and trends of performance testing programs in livestock. He mentioned that both improved management and increased effectiveness of selection have contributed to improvement resulting from the use of performance records. R. E. COMSTOCK of Minnesota stated that there had been little impact of modern breeding knowledge on swine breeding practices. He believed that breeds should be evaluated in terms of their combining ability, selection within breeds should be intensified using key herds for improvement, and breeds which are not honestly competitive should be discouraged in recommendations to breeders. He said that the three essentials of a breeding program were careful choice of foundation stocks, provisions for recombination of genes affecting economic traits, and methods for increasing frequency of useful genes. He asked if there was enough to be gained to warrant the introduction of exotic stocks. However, he believed the effort should be made to find out.

In Session Four, several new approaches to plant and animal improvement were presented. A. B. CHAPMAN of Wisconsin ably presented the case for checking the application of genetic theory to livestock by an intermediate step consisting of animal breeding research with laboratory animals. M. R. IRWIN and W. H. STONE, also of Wisconsin, showed the applications to livestock improvement made from findings of blood antigen research. They related the evidence that genes for controlling blood groups are not selectively neutral and stated that research is needed to study the association of blood types and other animal traits. Reporting on new research with plants, Q. JONES of the USDA presented an outline of the efforts being made to identify the major constituents of plants and seeds, so that their possible use in new industrial outlets may be determined. R. S. CALDECOTT of the USDA and Minnesota described the potential value of increasing genetic diversity in plant species through the use of ionizing radiations. He

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suggested that recurrent irradiation of animal species which are polyploids could result in sufficient genetic divergence in the irradiated lines so that F_1's between the irradiated line and the original progenitor would evidence hybrid vigor. From such F_1's, it should be possible to select true breeding lines. Climaxing the presentations on new approaches were R. I. SAILER's suggestions for improving the beneficial characteristics of insects. He pointed out that in addition to conventional uses of insects, they also served as natural enemies of insect pests and noxious weeds. He presented the evidence that useful insects should be subject to genetic improvement. W. V. LAMBERT of Nebraska emphasized the need for basic research in the sciences underlying the genetic improvement of plants and animals, as well as the need for discovery of new sources of germ plasm for food, fiber, and chemicals. He stated that there is need for the establishment of special centers for research in the various fields. Such centers would provide for a concentration of top scientific manpower and they would provide for most economic use of the expensive facilities needed for such research.

During the final session, E. JAMES of the National Seed Laboratory described the procedures of this laboratory which was established to provide seed storage for plant stocks. Seeds are accepted from federal, state, and private institutions and individuals based on their value as germ plasm for future use and comparison. Research workers in the United States, its territories, or possessions may receive seed from collections stored at the laboratory. R. E. LARSON of Pennsylvania presented the urgent need for establishing repositories or other means for preserving valuable germ plasm as vegetative stocks. Among the species which must be propagated clonally are deciduous fruits and nuts, subtropical and tropical materials, small fruits, sweet potatoes, and certain forage and fine turf. N. L. VANDEMARK of Illinois reviewed the progress made in preservation of animal germ plasm by freezing semen, chemical control of sperm metabolism, and lyophilization. He also mentioned the possibilities of storage of ova or ovarian and testicular tissue. F. B. HUTT of Cornell reported on the identification and elimination of defective animal germ plasm. He stated that the feasibility of increasing resistance to disease by breeding has been demonstrated with leucosis in fowls, and similar selection with other animals and other diseases seems desirable. T. C. BYERLY of the USDA closed the discussions with mention of the need to evaluate exotic stocks in the countries where they now exist. He stated that increased emphasis should be placed on methods and facilities for storaging of germ plasm of both plants and animals, including propagative plant stocks.

The symposium was organized by R. E. HODGSON, Animal Husbandry Research Division, ARS, USDA, Chairman of Section O: Agriculture, and Vice-President of A.D.S.A. He was assisted by a group of specialists in the Agricultural Research Service, USDA, and H. B. SPRAGUE of Pennsylvania. C. O. ERLANSON, E. J. WARWICK, R. MAGRUDER of the USDA, and H. J. SLOAN of Minnesota and A. E. BELL of Purdue served as chairmen of the various sessions. The papers of the symposium are expected to be published in a single volume by A.A.A.S. Until the surplus supply of the abstracts of the papers is exhausted, copies may be obtained by writing R. E. Hodgson, Director, Animal Husbandry Research Division, ARS, USDA, Beltsville, Maryland.

—J. W. THOMAS
A.D.S.A. Representative to A.A.A.S. Council, Animal Husbandry Research Division, Beltsville, Maryland

Food and Drug Administration Requirements

How to meet the requirements of the Federal Food, Drug, and Cosmetic Act for cream and butter is outlined in Leaflet No. 9 from the Food and Drug Administration. It outlines the responsibility of the producer and of the milk plant operator. This pamphlet gives a brief résumé of the application of the law to dairy products, general information on sanitation, practical information and instruction on the prevention of contamination of dairy products with antibiotics and pesticides. The cost of this pamphlet is 10c, with a 25% discount on orders of 100 or more.

Completed Thesis

M.S. Degree

G. C. VAN DAM—Instrumental methods for determination of bull sperm motility. University of Illinois, Urbana.

Dairy Technology Societies

Atlanta—The February meeting was a special program, to which were invited public health officials, sanitarians, food and drug administration and department of agriculture personnel, and others involved with the inspection and sanitation of milk and food establishments. Speaker was WILLIAM HICKEY, representative of the public health committee of the Paper Cup and Container Institute, New York City, and president of the International Association of Milk and Food Sanitarians. Subject: Ethics in Milk and Food Inspection Programs.

Central Illinois—PAUL STEINKE, Paul-Lewis Laboratories, Inc., Milwaukee, Wisconsin, was the speaker at the February meeting, in the

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Illinois Hotel, Bloomington. His subject was Bacteriophage in the Dairy Industry.

New members welcomed recently were: D. L. HELM, Beatrice Foods Company, Decatur, and R. H. WEANER, Peoria Health Department, Peoria. Quite a few members were reinstated, also.

Detroit—Cregars Pickwick House was the locale of the February meeting. FRANK KOVAL, Executive Secretary of the Michigan Dairy Products Association, spoke on The Effect of Modern Dairy Industry Practices on Milk Flavor Quality.

Kansas City Area—This group's fourth program of the year—January 28—featured U. G. GRAYSON, Administrator, Kansas City Milk Area, Kansas City, Missouri, as guest speaker. His subject: Marketing Order and What It Means. Stated purpose of this group is: To render a service to all men and women employed in milk and milk products of that area and to consider problems which will assist all of them in their efforts to furnish the consuming public with milk and milk products of high quality.

Maine—Ninety-four persons attended the midwinter meeting of this society, held at the University of Maine at Orono. The meeting included a tour of the University barns and inspection of the pipeline system, a panel on pipeline milkers, composed of A. C. GUSTAFSON of Chore Boy Mfg. Co., KENNETH MARQUIS of Babson Bros. Company, O. E. WILDER, De Laval Separator Company, and F. A. E. SMITH of Cowles Chemical Company, and E. A. QUIST of Corning Glass, and a talk by J. F. WITTER, Professor of Veterinary Science, University of Maine, on Why Antibiotics?

Metropolitan—Topic of February's cocktail-dinner-meeting was: A Novel Approach to Chemical Analysis, in which E. W. CATANZARO, research chemist, Technicon Instruments Company, touched upon the latest aspects of pesticides, antibiotics, and butterfat analyses, utilizing the auto-analyzer. New members welcomed: E. A. QUIST, Corning Glass Works, JOHN RAHT, Eastern Crown Corporation, and WM. HALSTEAD, Oakite Corporation.

Minnesota—Materials Handling was the featured topic at the February meeting at Worwa's Cafe, and R. T. TURNBULL, Cherry-Burrell Corporation, was the speaker.

North Carolina—CHARLES WEINREICH, research director, Cherry-Burrell Corporation of Cedar Rapids, Iowa, featured the topic: What's New in Dairy Equipment? at the group's February dinner meeting. The talk included information on freezers, automation of all kinds, and ultra-high-temperature equipment.

Philadelphia—FRANK MARTIN'S talk at the February 11 meeting brought out high lights of his recent stay in India, Pakistan, and Burma, as a representative of the Dairy Society International and the Foreign Agriculture Service of the UDSA. Mr. Martin, treasurer of Martin Century Farms Milk Company, Lansdale, and Martin-Witchwood Ice Cream Company, Springhouse, Pennsylvania, with a nutritional chemist, explored food research projects in these countries which might overcome the tremendous protein deficiency in the diets of the populations there.

Tri-Cities—February meeting featured both a speaker—DON EAKLE, director of Culture Department, Chr. Hansen's Laboratory, Inc., on Freeze-Dried Cultures, Their Manufacture, and Use in the Industry—and a film—Success or Failure. Those attending were urged to bring problems concerning cultured products and to be prepared to ask questions.

Utah—January meeting was held on the 18th in Salt Lake City, with MYRON HALES, Klenzade Products, Inc., speaking on Cultures and Cultured Products. Thirty-nine members and guests were present. The third annual meeting of the group is to be held in conjunction with the Annual Dairy Manufacturers Short Course, March 1-3, at Utah State University, Logan.

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JOURNAL OF THE BRITISH SOCIETY OF ANIMAL PRODUCTION

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CRICHTON, AITKEN & BOYNE: Effect of Plane of Nutrition on Dairy Cattle.

This journal is a continuation and expansion of the *Proceedings of the British Society of Animal Production* (1951-8) and appears twice a year in March and September. The annual subscription is 30s. ($5.00 in U.S.A. and Canada), single parts 17s.6d. ($2.75 in U.S.A. and Canada) net. Orders and subscriptions should be sent to:—

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STUDENT CHAPTER NEWS

J. E. JOHNSTON, Editor

A Section Devoted to News of Student Members

Annual Meeting Program for the Student Branch Section of A.D.S.A.

The first annual meeting of the Student Branch Section of the American Dairy Science Association will be held in 1960 at Logan, Utah. The program has been arranged by a special committee for the mornings of June 20, 21, and 22. The primary purpose of this program is to establish the framework for a national organization that will serve the student affiliate members and especially the 41 student affiliate chapters.

The Student Branch of A.D.S.A. is new. Final approval became a reality with the affirmative vote of the membership of A.D.S.A. for a change in the constitution that will permit the formation of the Student Branch. Prior to this year we were operating as a committee and had established two award programs, the Student Chapter Award and the Student Graduate Award. These will be continued similarly to last year.

It is important that all student chapters have a representative at the 1960 annual meeting. A special committee consisting of REAVES, RUDNICK, MICKLE, and WILLIAMS has been working for several months to develop the type of program they think will best serve the needs of the students. They have rewritten the constitution which will be presented and perhaps some of you may want to consider this a constitutional convention. At any rate, we must have some rules and by-laws to work with in the form of a constitution. Above all, we want the national Student Branch to be an organization of the students, governed by and for them. We realize that in many cases the faculty advisor may be the spokesman for his chapter.

OSC Dairy Club

The Oregon State College Dairy Club is again this year an affiliate member of the A.D.S.A. This club serves a unique function on the OSC campus, in that it draws members from two departments. The dairy technology students are members of the Food and Dairy Tech Department, which has a Food Tech Club, while the dairy production boys are under the Dairy and Animal Husbandry Department, in which there is a Withycombe Club for animal husbandry students.

The club members feel this is an asset to them, in that they have more chance to view problems and advantages of both sides of the dairy field. It opens the door to a deeper understanding of their industrial cousins' behavior on current issues and hot dairy topics such as the milk antibiotic problem.

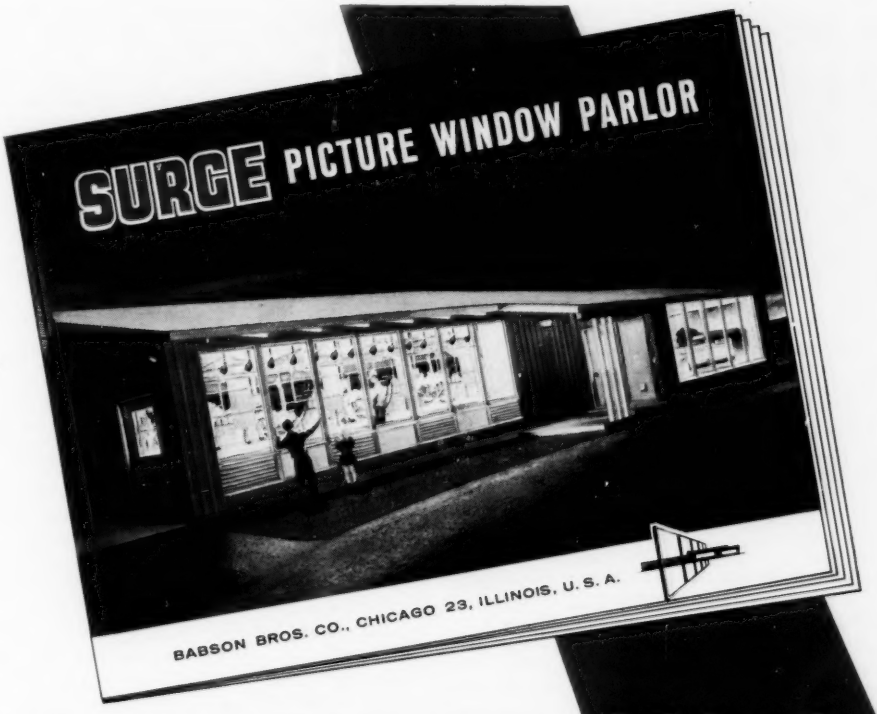
Judging competition. Being made up of both dairy production and dairy processing boys, the club participates in both dairy cattle and dairy products judging. In September, the dairy cattle judging team wanted to attend the national contest in Waterloo, Iowa, so they began soliciting funds. An employer of one of the boys, W. J. WINEBERG, Orchards, Washington, sponsored the trip by paying for round-trip plane tickets for the three team members and an alternate. The team returned with fifth place and fifth high man in the contest.

The dairy club paid team expenses for dairy products and dairy cattle judging teams to compete in the Pacific International contests. The products team placed second in Cheddar cheese and butter and first in ice cream, to put OSC in second place. Although the cattle team placed third in oral reasons and Holstein competition, and piled up first in Guernsey, Ayrshire, and Jersey judging, they were edged to fourth place in the contest.

The products team called upon the Oregon Dairy Industries for assistance when they began preparing for the National Invitational Collegiate Dairy Products Judging Contest in Chicago, December 1. ODI gave each of the three team members $200 for trip expenses. The team—two boys and a girl—the first girl ever to compete in this contest—took second in Cheddar cheese and ice cream, placing it fourth in the competition.

Club activities. The club is going strong again this year on coffee and Nutty Buddy sales. Nutty Buddies are the drumstick type ice cream—cone snack that the members make in the college dairy plant and sell at the dairy bar and to the dormitories for Saturday lunch dessert. This type of activity lets the boys get better acquainted by working together. They serve coffee and milk at several conventions on the OSC campus and will maintain a smorgasbord for the three-day February ODI meeting.

Coming during spring vacation is the club-sponsored annual spring field trip. This year the boys plan to go to Los Angeles to visit some of the Southern Cal dairy farms and processing plants. Half the trip expenses are generally furnished through the club treasury. This is the profit the boys get from contributing free time to the Nutty Buddy operation and to the care of the club dairy cow.



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Pictured above, talking with I. A. Gould, Chairman of the Department of Dairy Technology, are, from left to right: Dennis Heldman, winner of the Virginia Dare Award; Paul Culler, winner of the Annual Ohio Dairy Boosters Association Scholarship; and Ed Kysilka, winner of the Robert B. Stoltz prize.

Edward Kysilka was selected as the recipient of the annual Robert B. Stoltz Prize for scholarship and leadership in Dairy Technology. This consists of a cash award of $300. He has been unusually active in extra-curricular activities in the Student Dairy Club and the Dairy Tech Teamsters and has self-financed more than 50% of his college expenses. He is married and is a student affiliate member of the national professional organization, the American Dairy Science Association. He has received a previous scholarship from the Northeastern Ohio Dairy Technology Society.

Paul Culler was awarded the Annual Ohio Dairy Boosters Association Scholarship of $150 for his participation in extracurricular activities and leadership abilities. He is past-president of the Dairy Tech Student Club, is secretary of Ohio Staters, Inc., and served as secretary-treasurer of the Dairy Tech Toastmasters. He is a student affiliate member of the national professional organization—the American Dairy Science Association. He was a member of this past year's judging team which won first place in milk judging in the International Dairy Products Judging Contest and was awarded a silver medal for placing second in cheese judging in competition with 71 other contestants.

Dennis Heldman was awarded the Virginia Dare Award because of outstanding performance in the area of the ice cream industry. This award involved a plaque and a cash prize of $25. He is president of the Dairy Tech Toastmasters Club, has demonstrated superior scholarship, was awarded the silver medal for his performance in ice cream judging in the International Dairy Products Judging Contest held last fall in Miami Beach, Florida.

The Stoltz Award to Kysilka and the Boosters Award to Culler were made at the banquet held in connection with the Annual Meeting of the Ohio Dairy Products Association scheduled for January 26.

The Ohio State University Dairy Tech News—Fall Quarter

The Ohio State Dairy Tech Club officers for the 1959-60 school year include Ed Stagney, president; Keith Jenkins, secretary; and Roger Wolf, treasurer. Keith Jenkins serves as reporter for the Journal of Dairy Science.

The first activity of the 1959-60 school year was the annual Bean Feed, the event at which the freshmen in Dairy Tech are introduced to the faculty and upperclassmen. Because of inclement weather, the scheduled football game which would have pitted the freshmen against the upperclassmen had to be cancelled. Instead, several films, one of which told about the Swedish Dairy Industry, were shown. Afterwards dinner, consisting of baked beans, wieners, salad, milk, and ice cream, was served. Later in the evening, the members of the 1960 Dairy Products Judging Team were introduced, and the awards and medals they had won at the International Collegiate Students Judging Contest were shown. After a few words from Dr. Gould, Chairman of the Department of Dairy Technology, and Ed Stagney, president of the Dairy Tech Club, the activities were called to a halt.

Other club activities during the fall quarter have included the club serving as co-host with the department for the Annual Dairy Technology Homecoming Brunch on Saturday, October 17. The club assisted in entertaining alumni and friends, and with the brunch arrangement and food service. After the meal almost everyone attended the Ohio State–Purdue football game. In November, the Dairy Tech Club held a hayride for all club members, at Duro's Dude Ranch. There were approximately twenty-five couples in attendance.

The club again sponsored a Christmas Cheese Sale, with Ron Klausing serving as chairman. About 160 boxes of cheese were sold and the club realized a handsome profit. Ron was singled out for his terrific job in handling and promoting the activity.

Among the activities scheduled for the Winter Quarter will be the Annual Dinner Dance, sponsored jointly by the Dairy Tech and Dairy Science Clubs.

Three senior students in Dairy Technology have been honored for their achievements by being selected for special scholarship awards. They are E. T. Kysilka of Cleveland, P. L. Culler of Lucas, and D. R. Heldman of Columbus.

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RECONSTITUTABILITY OF DRIED WHOLE MILK. I. THE EFFECT ON SINKABILITY OF THE MANNER OF HANDLING FRESHLY DRIED MILK[1,2]

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SUMMARY

A test has been devised which adequately measures the variations in the sinkability of dried whole milk, a characteristic related to reconstitutability. The sinkability of the dried milk was greatly affected by the manner in which the dried milk was handled after drying. Maximum sinkabilities were maintained when the dried milk was well chilled before the dried milk particles were allowed to rub against each other or any object. A temperature-conditioning treatment was developed which allowed the recovery of the initial sinkability possessed immediately after drying. This phenomenon was attributed to the physical arrangement of the glycerides of the surface fat on the dried milk particles.

Recently, considerable attention has been focused on a new approach to the art of drying whole milk. Sinnamon *et al.* (6) reported the properties of a dry whole milk which had been dried under a high vacuum in the form of an expanded sponge-like structure. After a light crushing of the dried milk structure, the dried milk dispersed easily in cold water. Previously, Kielsmeier (2) developed a method of drying milk in films under high vacuum. He reported that after the dried milk films were crushed the product had better cold water reconstitutability than commercial spray-dried whole milk. However, Kielsmeier (3) noted that while the dried milk wetted and sank quickly from a quiescent water surface shortly after drying, it lost much of this desirable property after 24 hr. of storage at room or refrigerated temperatures.

The purpose of this paper is to report a method of handling the dried milk, which was developed by Kielsmeier, in such a manner that it will retain or regain the wettable and sinkable characteristics possessed immediately after drying. An explanation of the observed phenomena also will be presented. The dried milk was produced by drying, under a vacuum of 4.8 mm. Hg $\pm$ 0.2, a 35% total solids milk concentrate. The drying was done in flat pans, and the finished dried milk had a film structure. If the concentrate was gassed prior to drying, the final dried films were extremely thin (from the fine foam structure). With no gassing, the finished product was in coarse, thick films. Both dried milks were consistently soluble to the extent of 99.98% or better, as indicated by a solubility index (1) of 0.01 to 0.02 ml. Therefore, the problem was not one of improving the final solubility, but of improving or maintaining the

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[2] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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characteristic which allows the dried milk to wet and sink more quickly, thereby permitting the dried milk to disperse in a shorter period of time.

The Sinkability Test. It was necessary to have a test which could be used to evaluate the wettable and sinkable characteristics of the dried whole milk. A review of the literature disclosed no test which was adequate to measure these characteristics. Therefore, a test was devised specifically for this purpose. The test determined the rate at which the dried milk sank into a quiescent water medium and it was therefore designated the "Sinkability Test."

Materials. The materials required to carry out the test are shown in Figure 1 and include:

A. Funnel-shaped paper extension to aid in guiding the dried milk into the separatory-type vessel.

B. A separatory-type vessel made from a 5- by 40-cm. test tube which was cut down to a length of 9 cm. A glass tube outlet 1 cm. in diameter was fused into the bottom and reduced to a length of 1 cm. To this was fitted a short rubber tube.

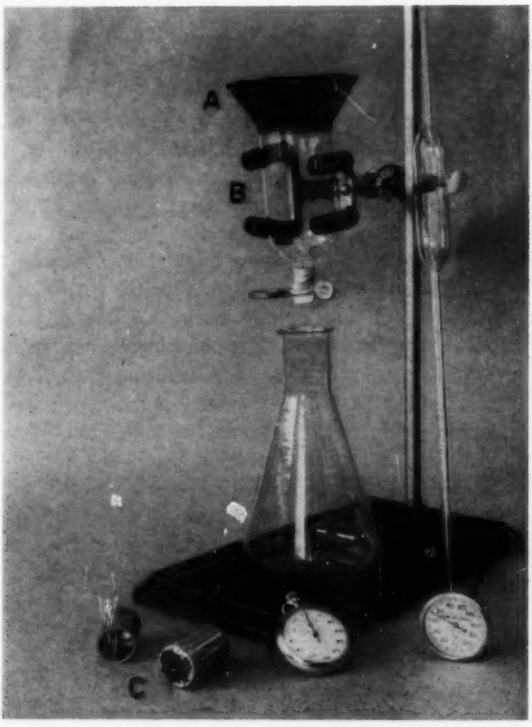

FIG. 1. Materials required for the sinkability test: A—Funnel-shaped extension to aid in guiding dried milk into separatory-type vessel; B—Separatory-type vessel; C—Telescoping thimble to sprinkle dried milk on water surface.

C. A stainless steel thimble 2.5 cm. in diameter and 3.5 cm. deep. A metal sleeve, with a piece of screen soldered on one end, which would telescope over the open end of the thimble.

Method. Five hundred milligrams of dried milk were weighed into the thimble. Fifty milliliters of distilled water at 25° C. were placed in a separatory vessel with a pinch clamp in place. The 500-ml. Erlenmeyer flask was positioned beneath the outlet. The dried milk was sprinkled quickly and evenly on to the quiescent water surface; the stop watch was started at the time the sample first struck the water surface. After a predetermined time interval, usually 20 sec. to 3 min., the pinch clamp was opened and that portion of the dried milk which had sunk was drawn off quickly with the water into the Erlenmeyer flask, leaving behind that fraction of the sample which had not sunk. After complete solution of the sample in the flask, a 5-ml. aliquot was removed and total solids were determined by the Mojonnier method (5), to ascertain the fraction of the sample which sank. The result was expressed as per cent sinkability and was calculated as follows:

$$\text{Per cent sinkability} = \frac{\text{weight of solids in 5-ml. aliquot} \times 50 \times 100}{\text{weight of original sample} \times 5}$$

Consideration of variables in the Sinkability Test. Before standard conditions for the sinkability test were established, the effects of certain variables were studied.

(a) *The time interval employed in the test.* The time interval for contact of the dried milk with the water was not standardized, but was changed to suit the degree of sinkability of the dried milk being tested. The time interval allowed for dried milk having relatively poor sinkability was as long as 3 min., and as short as 20 sec. for dried milk having excellent sinkability. For this reason, the test time (time the sample was allowed to remain in contact with the water during the test) will be stated with all results.

(b) *Dried milk temperature.* The dried milk temperatures were found not to be critical at the temperatures normally encountered during testing. No significant difference was noted when samples of a lot of dried milk were immediately tested after removal from storages ranging in temperature from 3 to 21° C. It was, therefore, considered unnecessary to temper the dried milk to a common temperature for the sinkability test. However, refrigerated samples were allowed to remain at room temperature in sealed containers for about 5 min. before weighing, to minimize the condensation of moisture on the samples.

(c) *Water temperature.* The temperature of the water used in the test was a critical factor for the sinkability determinations. An increase in the water temperature caused the sinkability values also to increase. For convenience, the water temperature was standardized at 25° C. The use of this temperature also minimized any changes in the water temperature during testing, as the temperature of the laboratory was usually 25 ± 2° C.

(d) *The dried milk particle size.* Studies showed the dried milk particle size to be quite significant. The smaller the particle size, the lower were the sink-

ability values. The particle size range was, therefore, standardized by scraping the dried milk films from the drying pans, crushing them, and sieving the dried milk particles through two screens, with 1.5 mm. and 210 μ openings, respectively. The portion which passed through the coarse screen but did not pass through the finer screen was used for testing. The remainder was discarded.

(e) *The moisture content of the dried milk.* The moisture content of the dried milk samples should be considered in calculating the sinkabilities. As an illustration, if a sample contained 3% moisture and was observed to sink completely, the calculated result would be only 97% sinkability, since 3% of the sample was water lost during the total solids determination. Therefore, the corrected sinkability for a dried milk containing 3% moisture was determined as follows:

$$\text{Corrected sinkability} = \text{Calculated sinkability} \times 1.03$$

In this work, the dried milks tested had a moisture content of $3 \pm 0.5\%$. Therefore, all values reported have been corrected on the basis of a 3% moisture content, as any error incurred was less than the accuracy of the test. Replicate determinations on samples of dried milk did not vary more than 2.5 percentage points. The variation was usually much less than this.

Effect on sinkability of the manner of handling dried milk after removal from the drier. Preliminary experiments clearly indicated that there was little or no difference in the sinkability of freshly dried milk, whether it was chilled immediately after removal from the dryer and before crushing and sizing, crushed before chilling and sizing, or held 24 hr. at room temperature before chilling, crushing, and sizing. However, when the dried milk was crushed and then held 24 hr. at room temperature, a decided loss of sinkability occurred.

In another experiment, the results of which are presented in Table 1, the effect of a delay in chilling the dried milk after sizing is clearly demonstrated. Freshly dried milk was crushed immediately after removal from the dryer and divided into two lots. One-half minute after crushing, Sample A was placed in an aluminum foil envelope on a freezing shelf and then sized after 60 min. of chilling. The remainder of the dried milk, Sample Series B, was sized and divided into eight portions. Each portion was sealed in an aluminum foil envelope and held at room temperature for time intervals of ½, 2, 7, 12, 30, 60, 120, and 360

TABLE 1

Effect on sinkability of delayed chilling after crushing and sizing the dried milk

Sample	Time interval between sizing and chilling	Sinkability (20-sec. test time)
	(min.)	*(%)*
A (chilled before sizing)		77
B_1	½	44
B_2	2	42
B_3	7	41
B_4	12	38
B_5	30	23
B_6	60	24
B_7	120	22
B_8	360	23

min. before being placed on a freezing shelf. All samples were then held 20 hr. at −22° C., after which the sinkabilities were determined, using a 20-sec. test time.

It was apparent that the most critical factor in handling film-dried milk after drying was the sequence of crushing, sizing, and chilling the dried milk. The best results were obtained when chilling preceded the crushing and sizing of the dried milk. Sizing before chilling was very detrimental to the sinkability of the dried milk. It must be assumed, therefore, that the manner in which the dried milk is handled in a commercial operation could be very critical. For example, if the crushed dried milk were conveyed by a screw-type conveyor or by a vacuum pipeline before cooling, the particles would receive considerable abrasive action, which might cause the same loss of sinkability as that caused by sizing.

To simulate such conditions, a lot of freshly dried milk was lightly crushed and divided into two samples, A and B. Sample A was chilled and set aside, whereas Sample B was subdivided among five glass sample jars. These jars were immediately placed on a wrist-action shaker at room temperature for periods of time varying from 0 to 180 sec. The shaking action caused the dried milk particles to be thrown against each other. After removal from the shaker the samples were quickly chilled for 60 min., then sized. After 20 hr. of refrigerated storage, sinkabilities were determined, using a 20-sec. test time.

Sample A which was chilled for 20 hr. in a room at −22° C. was also subdivided among five sample jars. These jars were placed on a wrist-action shaker and handled in a manner similar to Sample B. The samples were removed from the shaker, chilled again for 60 min. to ensure that the fat was in a solid state, then sized. The sinkability results are presented in Table 2.

The agitation of the warm dried milk caused a great loss of sinkability, but when the dried milk was previously chilled only a slight loss occurred. The difference between the per cent sinkabilities of the warm and cold samples which received no agitation appeared to be significant. This may be explained by the fact that the warm dried milk was unavoidably agitated when it was divided and placed in the five sample jars. The cold sample, however, was divided after chilling.

Temperature conditioning of dried milk. It has been shown that excellent sinkabilities can be maintained when dried milk is chilled immediately after removal from the dryer. It was thought that this was related to the solidification of the surface fat before its physical arrangement was altered. It seemed reason-

TABLE 2

Effect on sinkability when dried milk was agitated before and after chilling

Agitation time	Samples agitated warm	Samples agitated cold
(sec.)		
0	67	74
15	53	75
30	46	74
60	32	72
180	12	71

able to assume, therefore, that if dried milk, which had lost much of its excellent sinkability, were warmed to a temperature above the melting range of milk fat, then chilled without agitation, the optimum physical arrangement of the surface fat would be regained, and the excellent sinkability which the dried milk possessed immediately after drying might be recovered. This process of warming and cooling the dried milk under controlled conditions will hereafter be referred to as temperature conditioning.

In a temperature-conditioning experiment typical of many that were conducted, the sinkability of a freshly dried sample of film-dried milk was found to be 70%. After 20 hr. of storage at 6° C. the value decreased to 38%. Following temperature conditioning, which involved heating the dried milk for 2 hr. at 50° C. and chilling immediately on a freezing shelf for 20 hr., the sinkability was increased from 38 to 82%. The effect of temperature conditioning on the sinkability of the dried milk is illustrated in Figure 2. Similar trials with film-dried skimmilk showed no change in the sinkability after temperature conditioning. Thus, it appeared that temperature conditioning of film-dried whole milk caused some change to occur in the fat of the dried milk which allowed the initial sinkability to be regained. Investigations were carried out to determine the minimum heat treatment of the dried milk required to obtain a maximum sinkability.

A time-temperature relationship for temperature conditioning. By experimentally determining the time required to reach a given per cent sinkability for a range of temperatures from 41 to 60° C., it was possible to develop a time-

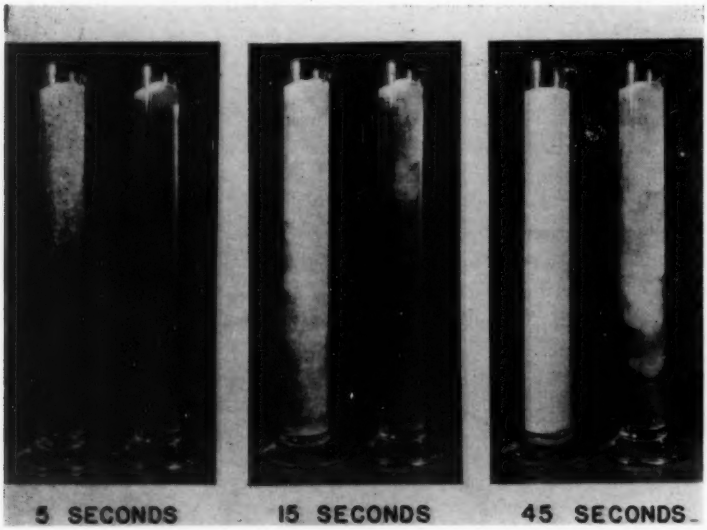

FIG. 2. A comparison of the sinkability of dried milk before temperature conditioning (right-hand 250-ml. graduated cylinder) and after temperature conditioning (left-hand 250-ml. graduated cylinder), at 5, 15, and 45 sec. after being placed on the water surface.

temperature curve. This curve could be used to predict the time required to treat dried milk at any suitable temperature under the conditions of the experiment. To simulate possible commercial conditions of temperature conditioning on a small scale, the dried milk was placed on a 7- by 12-cm. aluminum foil–covered transite platform to a depth of $\frac{1}{8}$-in. The platform was placed in a tunnel through which heated air was passed at the desired temperature to heat the dried milk. This temperature was measured by a thermocouple junction projecting $\frac{1}{16}$-in. up through the center of the platform and into the dried milk. As soon as the heating was completed, the dried milk was quickly transferred to aluminum foil envelopes which were immediately sealed and placed on a freezing shelf to effect rapid cooling of the dried milk. After 20 hr. of chilling the sinkabilities were determined, using a 30-sec. test time. By plotting the logarithms of the times required to reach 70% sinkability (an arbitrary figure) against the heating temperatures, a curve as shown in Figure 3 was obtained which was mathematically expressed by the following equation:

$$\text{Temperature } (^\circ \text{C.}) = 59 - 16.32 \log \text{time (minutes)}.$$

The labile character of sinkability. In the previous experiments no improvement in the sinkability occurred until the heating temperature exceeded 32° C. Therefore, a twofold experiment was conducted which determined the effect of a 32° C. storage temperature on temperature-conditioned dried milk, and also the effects of a cyclic temperature treatment. This was accomplished by heating about 75 g. of dried milk in a glass sample jar at 50° C. for 4 hr., followed by rapid chilling at 6° C. for 20 hr. After the sinkability was determined, the jar of

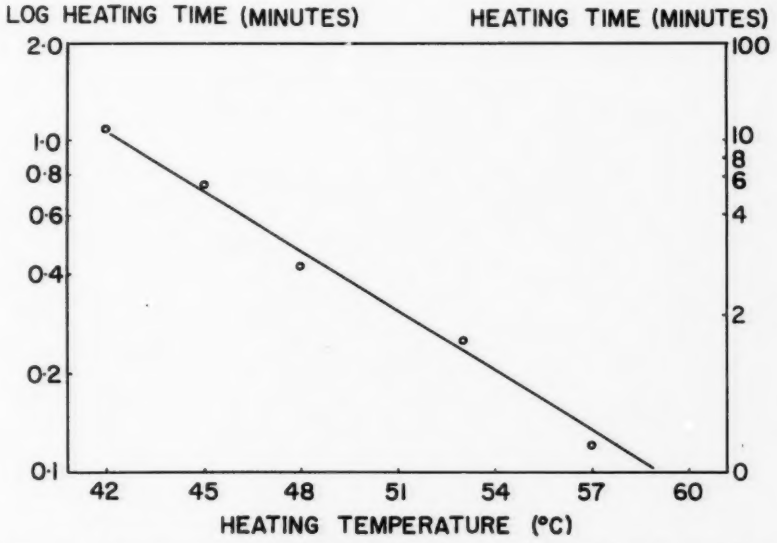

FIG. 3. A time-temperature relationship for temperature conditioning dried milk under standard conditions.

dried milk was held for 4 hr. at 32° C. Using the same sample of dried milk, the complete procedure was repeated twice, followed by a final temperature-conditioning treatment. Sinkabilities were determined after every step, using a 1-min. test time. The results are presented in Table 3. It was evident that the increased sinkability obtained by temperature-conditioning dried milk was a labile characteristic at 32° C. It was also very clear that the process whereby the characteristic was gained or lost was reversible.

TABLE 3
Effect of a cyclic temperature treatment on the sinkability of a dried whole milk sample

Sequence of steps	Temperature treatment of dried whole milk	Sinkability (1 min. test time)
		(%)
1	Held 4 hr. at 50° C., and then chilled for 20 hr. at 6° C.	96
2	Held 4 hr. at 32° C.	55
3	Step 1 repeated	92
4	Held 4 hr. at 32° C.	53
5	Step 1 repeated	92

The stability of the sinkability property in storage. It has been shown (in Table 3) that a storage temperature of 32° C. will cause a considerable loss of sinkability in 4 hr. An experiment was conducted to determine the range of temperatures which would allow the sinkability of temperature-conditioned dried milk to diminish. A lot of film-dried milk was temperature-conditioned and stored at 6° C. Two-gram samples of the dried milk were then held at temperatures of 4, 21, 26, 30, 34, 36, 41, and 45° C. for 6 hr. The samples were then held 30 min. at 25° C., to minimize the differences in the dried milk temperatures before determining the sinkabilities. A 1-min. test time was used. The results, presented in Figure 4, indicated there was a considerable loss of sinkability between 30 and 36° C. It was, therefore, apparent that a storage temperature of 30 to 36° C. would be unsuitable.

Of more practical significance was the effect on sinkability of longer periods of storage. Samples of dried milk were stored at temperatures ranging from 4 to 24° C. The dried milk was preweighed in 500-mg. lots, placed in aluminum foil thimbles, sealed, and temperature-conditioned at 50° C. for 30 min., followed by rapid chilling and storage on a freezing shelf for 20 hr. Nine thimbles were placed in each of six glass jars, which were then held in storage at 4, 9, 13, 18, 21, and 24° C. for 22 days. Periodically, thimbles were removed and sinkabilities determined immediately, using a 20-sec. test time. The results indicated that storage temperatures of 24 and 21° C. were unsatisfactory, as the sinkabilities diminished considerably. Lower temperatures which were used appeared to be satisfactory, as high levels of sinkability were maintained. To determine more accurately the temperature at which the sinkability declined, data were taken from this experiment and plotted as in Figure 5. The sinkabilities of the dried milk at one day of storage were plotted against storage temperature. A curve was obtained which indicated a gradual decrease in sinkability when the

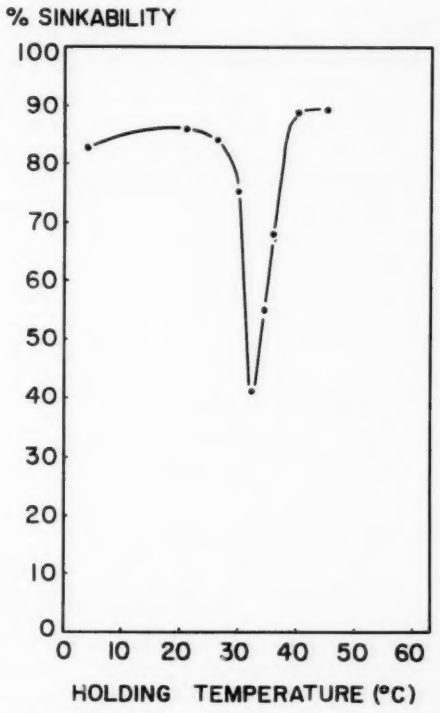

FIG. 4. Effect of a 6-hr. holding period at various temperatures on the sinkability of the temperature-conditioned dried milk.

storage temperatures exceeded 18° C. Sinkabilities for the storage periods of five and 22 days were plotted in a similar manner. It was observed that the sinkabilities decreased markedly in each case when the storage temperatures exceeded 18° C. Thus, it appeared that storage temperatures of 19° C. and above were unsuitable for prolonged storage.

Effect of temperature conditioning on the sinkability of other types of dried whole milk. The investigations reported thus far have been directed toward milk dried in films under high vacuum. Whenever the opportunity was presented, other types of dried whole milk were temperature-conditioned to ascertain the effect of such a treatment on sinkability. The results are presented in Table 4 and include a film-dried milk which had remained at room temperature for 2 yr. Without exception, every sample gave some measure of response to temperature conditioning. Roller-dried milk gave the least response; the commercial spray-dried milk showed a fair response, and the remaining samples showed a considerable improvement. Except for the roller-dried milk, the stability of the improved sinkability property appeared to be excellent during prolonged storage at 6° C.

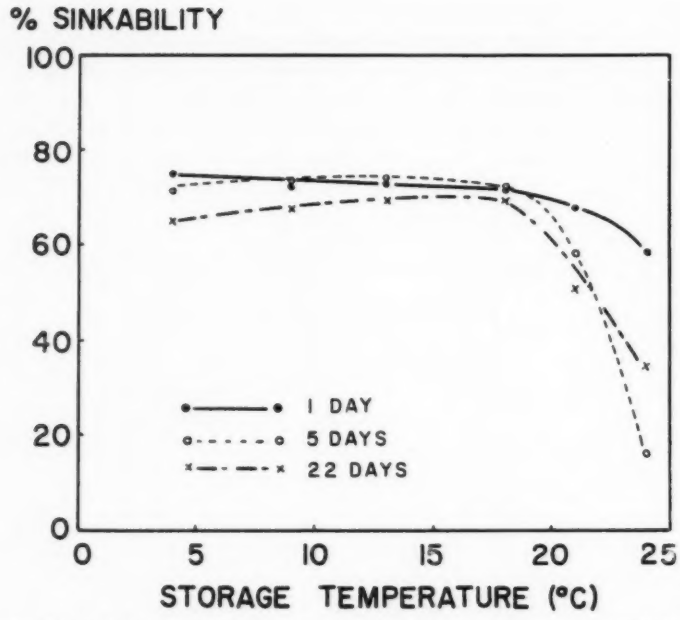

FIG. 5. Effect of storage temperatures on the sinkability of temperature-conditioned dried milk at one, five, and 22 days of storage.

TABLE 4
Sinkability of dried milks before and after temperature conditioning (TC)

Product	Test time	Sinkability		
		Before TC	After TC	
			20 hr.	Extended storage at 60° C.
	(sec.)	*(%)*		
Commercial spray-dried whole milk	60	10	41	40 (12 wk.)
Experimental spray-dried whole milk	60	31	90	83 (12 wk.)
Experimental aggregrated spray-dried whole milk	60	12	96	92 (18 wk.)
Commercial aggregated spray-dried whole milk	20	17	70	
Modified (lactose-enriched) dried whole milk	30	27	98	87 (26 wk.)
Thin film-dried whole milk (2 yr. at room temperature)	30	11	64	63 (12 wk.)
Roller-dried whole milk	60	7	17	5 (26 wk.)

Effect of the rate of cooling on the sinkability of heated dried milk. To determine the effect on sinkability of the rate of cooling heated dried milk, an experiment was conducted in which warmed samples of dried milk were cooled at varying rates. Only the times required to cool from 35 to 26° C. were considered, as it had been noted earlier that there was no effect on the sinkability of warm dried milk until the temperature reached 35° C. Also, the dried milk was rapidly chilled after reaching a temperature of 26° C. From the results presented in Table 5 it was observed that there was a correlation between cooling times and final sinkabilities. As the cooling times increased the sinkabilities decreased.

TABLE 5

Effect of the rate of cooling on the sinkability of dried milk which had been heated to 43° C.

Sample	Cooling time from 35 to 26° C.	Sinkability (30-sec. test time)
	(min.)	*(%)*
Control (chilled rapidly)		82
A	21	75
B	45	56
C	77	28

Effect of water temperature on the sinkability of dried milk before and after temperature conditioning. To better understand the changes which occurred during temperature conditioning, the effect of increasing the temperature of the reconstitution water on the sinkability of the dried milk before and after temperature conditioning was investigated. A lot of dried milk was tempered for 20 hr. at 32° C. to promote a minimum sinkability. The dried milk was then chilled for 20 hr. at 6° C. Using this dried milk and water ranging in temperatures from 6 to 40° C., sinkability values were determined, using a 20-sec. test time. A portion of the same dried milk was then temperature-conditioned (50° C. for 30 min., followed by rapid chilling) and stored for 20 hr. at 6° C. Sinkability values were then determined as before, using test water ranging in temperatures from 6 to 32° C. The results, shown in Figure 6, indicated that the water temperature was very critical between 20 and 25° C. for the temperature-conditioned dried milk (A), as there was a very sharp increase in the sinkability values in this range. The temperature range of 32 to 40° C. was critical for the dried milk which had not been temperature-conditioned (B).

Relationship of the melting range of fat in dried milk to the response to temperature conditioning. When dried milk was temperature-conditioned using a wide range of heating temperatures, there was a sharp increase in the subsequent sinkability between the heating temperatures of 38 and 41° C. It was apparent that this phenomenon might be closely related to the melting range of the milk fat. On this basis, it was predicted that a change in the melting range of the milk fat would cause a shift of the sharp break-point in the sinkability curve. For example, if the fat had a lower melting range the break-point would be at a lower temperature, but if the melting range were higher the break-point would occur at a higher temperature.

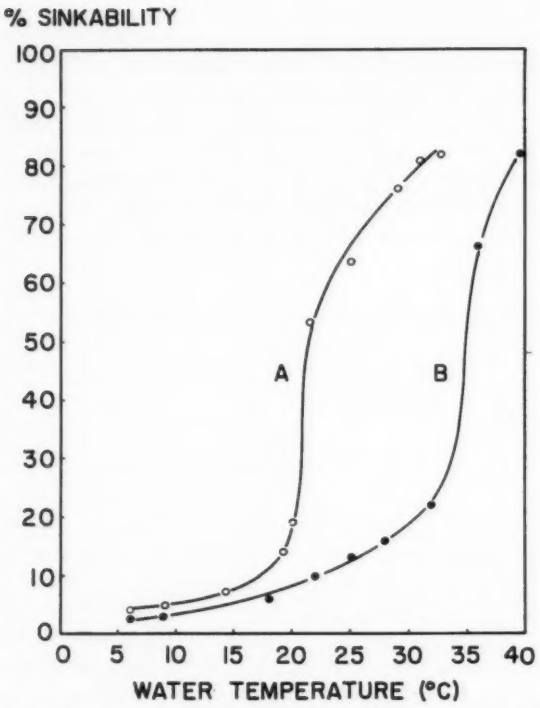

FIG. 6. Effect of the test-water temperature on the sinkability of dried milk after temperature conditioning (A) and before temperature conditioning (B).

An experiment was conducted in which milk concentrates were made which contained fats with wide melting-range differences. The fats were prepared by melting unsalted butter and decanting the butter oil after centrifugation. The serum was saved for later reconstitution purposes. The completely melted butter oil was placed in an incubator at 32° C. for 48 hr., after which the portion of the fat which had solidified was filtered off and set aside for use in a high-melting-point-fat concentrate. The oil filtrate was then held at 22° C. for 48 hr. and again filtered to remove the solidified fat. The solidified portion was discarded and the remaining butter oil was used in a low-melting-point-fat concentrate. This second separation was carried out to allow the two fat fractions to have distinctly different melting ranges. The melting range of the higher fraction, as judged visually by the capillary tube method, was 41 to 44° C., whereas the lower fraction was 12.5 to 13.5° C.

The two concentrates were made up using 33% condensed skimmilk, butter oil, butter serum, and sufficient water to standardize the solids-not-fat content at 25%. The fat test was 10.5%. The mixtures were homogenized at 67° C., using a two-stage homogenizer with pressures of 2,000 and 500 p.s.i. A control

concentrate was made by oiling-off similar unsalted butter (melting range 32 to 34° C.) and incorporating it into the condensed skimmilk as before. The concentrates were gassed and dried in the usual manner. Crushing and sizing of the dried milk was carried out immediately after drying, and sinkabilities were determined on this fresh dried milk about 30 min. later, using a 30-sec. test time.

A 2-g. sample of each lot was temperature-conditioned by heating at 50° C. for 15 min., followed by rapid chilling. After 20 hr. at 6° C. sinkabilities were determined as before. The results are presented in Table 6.

TABLE 6
Sinkabilities of three dried milks before and after temperature conditioning

Sample	Sinkability (30-sec. test time)	
	Fresh dried milk	Temperature-conditioned dried milk
	(%)	
Control	16	71
High-melting-point-fat dried milk	9	23
Low-melting-point-fat dried milk	81	82

It was noted that the high-melting-point-fat dried milk showed a low level of response to temperature conditioning. A range of temperatures up to 100° C. and a range of heating times up to 3 hr. were used, in an effort to obtain a greater response. In no instance did the response exceed a sinkability value of 23%. The low-melting-point-fat dried milk had excellent sinkability before and after temperature conditioning. The control responded normally to the temperature-conditioning treatment.

Samples of each lot were then held for 4 hr. at temperatures varying from 3 to 55° C. When temperatures above this range were used, holding times were reduced to as low as 10 min., to avoid protein denaturation. The sinkabilities were determined without cooling the dried milk. The test time was 30 sec. for the control and high-melting-point-fat dried milks and 15 sec. for the low-melting-point-fat dried milk. The reduction of the test time to 15 sec. was necessary, owing to the very high sinkability of the low-melting-point-fat dried milk.

The sinkability values were plotted against holding temperatures and a curve was obtained for each dried milk as shown in Figure 7. It was found that a sharp increase in the sinkability of the low-melting-point-fat dried milk occurred at about 29° C., whereas the increase of the control was at about 39° C. The sinkability of the high-melting-point-fat dried milk increased sharply at approximately 70° C. It is emphasized that these values were for dried milk which had not been chilled after being heated.

DISCUSSION

The manner in which the dried milk was handled immediately after drying had a very significant effect on the sinkability. The experimental results may be explained on the basis of the state of the surface fat. This surface fat appeared

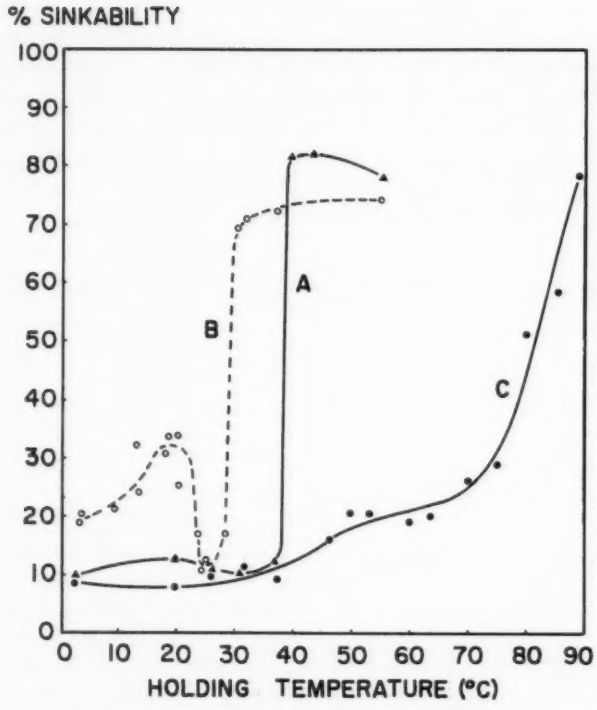

FIG. 7. Effect of various holding temperatures on the sinkability of three different dried milks: A—Dried milk containing unfractionated butter oil; B—Dried milk containing low-melting-temperature butter oil; C—Dried milk containing high-melting-temperature butter oil.

to arise mainly from the fractured edges of the dried milk particles. This is suggested by experimental results which showed practically no loss of sinkability in dried milk held in an uncrushed state at room temperature for 24 hr. However, when crushed dried milk was held 24 hr. at room temperature a sharp decline in the sinkability occurred. Apparently, 24 hr. was sufficient time for the fat, released by crushing, to migrate about the surfaces of the particles and increase their hydrophobic characteristics. Whenever unchilled dried milk was agitated, as in sizing, a loss of sinkability occurred. This may be explained as being due to the presence of liquid surface fat which was available to be transferred from particle to particle and thereby cause each particle to be covered by a greater area of fat. However, if the dried milk were chilled before being agitated the surface fat would be solidified and as such would not be readily transferred.

When the dried milk was handled or stored in such a manner that the sinkability diminished greatly, it was found that a temperature-conditioning treatment of the dried milk allowed the original sinkability to be recovered. The explanation of this phenomenon has not been definitely proven, but a postulation

is offered which appears to be credible. By subjecting the dried whole milk to the temperature-conditioning treatment it appears that the fat phase, and probably the fat on the surface, in particular, is adjusted in such a manner that it wets more freely. It is apparent that the heating of the dried milk during the temperature-conditioning treatment must be sufficient to melt the milk fat. It is postulated that this melting allows a redistribution of the glycerides of the milk fat to take place which may be similar to the distribution present when the warm dried milk is removed from the dryer. A rapid chilling of the dried milk may then have solidified the fat before this particular distribution was changed. However, if chilling proceeds slowly, the fat which solidifies may be definitely oriented and the random distribution of the glycerides will be altered. This postulation appears to be supported by the experimental evidence. For example, the initial sinkability of the dried milk was not regained until the heating temperatures exceeded temperatures which are known to be required to melt most of the glycerides of milk fat and the time at that temperature was enough to permit redistribution of the glycerides.

A clue to this glyceride redistribution may be found if a beaker of melted butter oil is held overnight at 32° C. Upon examination the following morning, one may find a partial solidification of the fat. Crystal-like structures are found clinging to the sides of the beaker; a considerable amount is present on the bottom; a few smaller structures are dispersed throughout the liquid fat; and, that which is most interesting, a relatively thick layer of solidified fat is present at the surface. This layer does not develop by bridging, but can be explained by a migration of the higher melting point glycerides to the surface from points within the body of the melted butter oil. Thus, a redistribution of the glycerides must have taken place. It is possible that this phenomenon may occur in the surface fat of the dried whole milk particles. If this were so, one would expect a thin film of high-melting-point glycerides to orientate at the air-fat interface of the free fat when the dried milk was held at 32° C.

The findings of King (4) may be considered as additional support for the postulation. King was able to show through a study of the birefringent layer on the fat globules in butter that some of the globules show the birefringent layer at the surface, and this layer consists of the higher melting point fats. To quote, "Hence, the molecules of the higher melting fats in the birefringent layer of the fat globules in butter must be orientated radially or perpendicular to the surface of the globule." King shows that since all globules in butter do not show this birefringent layer, its presence is dependent upon the exertion of pressure on the globules. The pressure in churning produces orientation. Since we have pressure exerted on the globules in drying, such orientation could be similarly induced.

Another factor should be considered before a full explanation of the observed phenomena is attempted. This involves the affinity of water for fat. If a glass slide is coated with a thin film of butter oil, chilled, and a drop of water placed on it, the water will exhibit very little affinity for the fat and will remain in a globular-like form. The angle of contact is large. However, as the fat film gradually melts, the water drop spreads out. The contact angle becomes small, showing lowered interfacial tension. This increased affinity of water for melted fat can also be demonstrated by placing water in a beaker which has been lined with a thin film of butter oil and chilled. While the fat remains

solid there is no visible meniscus, but after the fat melts a meniscus appears. Therefore, the state of the fat, whether liquid or solid, at the time of reconstitution may have a profound influence on such characteristics as wetting and sinking of dried milk.

A more complete explanation of temperature conditioning can now be offered. It has been noted that when dried milk was handled in a normal manner after drying, the sinkability diminished considerably after a short storage period. Since cooling was relatively slow, a redistribution of the glycerides may have occurred, with the result that a thin film of high-melting-point glycerides was formed at the air-fat interface of the surface fat on the dried milk particles. When this dried milk was subsequently placed on water at 25° C., the temperature was not sufficient to melt this film of high-melting-point glycerides, and complete wetting of the milk particle was prevented. However, after the dried milk was temperature-conditioned the surface fat was melted and the glycerides were rearranged in a random manner. Rapid chilling solidified the fat before orientation of the glycerides could occur. When the dried milk was then placed on water at 25° C. the water temperature was sufficient to cause the surface fat to partially melt and a more complete wetting of the dried milk particle occurred. The result was improved sinkability. This postulation would also explain the results presented in Figures 6 and 7 and in Table 6.

While this explanation of the postulation is based chiefly on results obtained from film-dried milk, it is probable that the response of conventionally dried whole milks to temperature conditioning and to warm reconstitution temperatures may be similarly explained.

ACKNOWLEDGMENT

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FACTORS AFFECTING THE DIRECT MICROSCOPIC CLUMP COUNT OF NONFAT DRY MILK

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SUMMARY

The relationship between the DMC of raw skimmilk and the DMC of the NDM made from it was found to vary widely within days and seasons of the year. The DMC per gram of the NDM ranged from 1.9 times to 71.0 times the DMC per milliliter of the skimmilk on 44 trials. The average increase was 9.1 times. Factors which were found to influence this relationship were: (a) The effect of breaking up of clumps due to agitation, (b) the removal of bacterial cells during the separation of the whole milk and subsequent clarification of the skimmilk, (c) the amount of heat treatment given the skimmilk during processing, (d) the length of time of cold storage of condensed milk prior to drying, and (e) the effect of drying.

In 1958, the United States Department of Agriculture (26) adopted as a standard for nonfat dry milk a maximum Direct Microscopic Clump Count of 300 million per gram. The Direct Microscopic Clump Count (DMC) was proposed as a means for improving the quality of nonfat dry milk. It was believed that faulty plant practices could be located and corrected by means of the DMC. It was also believed that there existed a sufficiently close relationship between the DMC of the raw milk and the DMC of the nonfat dry milk to warrant the conclusion that the standard was a reliable index of raw milk quality. It is the purpose of this paper to evaluate this conclusion and to assess the suitability of the DMC as a standard for grading purposes.

Supplee and Ashbaugh (24) apparently were the first to propose the direct count as a standard for nonfat dry milk, in 1922. Their work was done with roller-dried nonfat dry milk. Making a total of 12 runs, they reported a reduction of 28.4% in the DMC of the nonfat dry milk, compared with that of the original skim. Their results ranged from a decrease of 64.5% for Trial 7 to an increase of 93.1% for Trial 12.

At one time, many investigators believed that all the organisms which took a stain after pasteurization were viable. Hastings and Davenport (9), in 1920, studied the effect of pasteurization on the number of stainable bacteria after 24 hr. of storage at 50° F. In 13 out of 16 samples, reductions were found of between 10 and 30% of the original number of bacteria present. The type of organism present was reported to be of considerable significance.

Ward and Myers (27), using the Newman-Lampert stain, found a reduction of 86.1% in the DMC of milk held at 143° F. for 30 min. and a reduction of 97.3% after holding for 90 min. They therefore concluded that the bacteria which did stain were viable.

In 1942, Mickle and Borman (15) recommended the use of the DMC as a standard for pasteurized milk, based upon the assumption that the bacteria which are killed by pasteurization are usually rendered unstainable. They found the

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DMC to be a useful tool in detecting heat-resistant bacteria in the finished product, not found by the standard plate method. Robinton *et al.* (19) reported reductions in DMC's after heating at 80° C. for 30 min. on seven samples of raw milk ranging from 39 to 95%. Further decreases in counts were obtained on six of the seven samples after storing the milk at 8° C. for 48 hr.

Baker (3) studied the effect of pasteurizing and storage on the stainability of bacteria and concluded that the DMC on pasteurized milk may or may not be equal to a similar count on the same milk before pasteurization and in some cases may be only a small fraction of it. In a majority of the comparisons using *Streptococcus lactis* the pasteurized milk counts were less than 60% of the raw milk counts. *Streptococcus durans* stained well after pasteurization, but *Pseudomonas putrefaciens* showed especially poor staining qualities after pasteurization.

Although confusion has existed in the past, it is now widely recognized that most of the organisms which appear on a smear made from NDM are dead. Standard plate counts on NDM range from a few hundred to a few hundred thousand per gram, whereas the DMC may range from ten million to a billion.

The stain employed is an important factor affecting the level of the count obtained. Brandstein and Mundt (6) compared the bacteria counts obtained on raw milk using six different stains. Gray's stain yielded results 44% lower than the Breed Methylene Blue stain, and the Broadhurst and Paley stain (7) yielded results 66% higher than the Breed stain. The remaining three stains were within these limits. In a later modification of his stain, Gray (8) reported results ranging from 1.5 to 1.64 times higher than Breed's Methylene Blue Stain.

Olson and Black (18) reported on a comparative study of stains proposed for the DMC of milk. On approximately 420 samples the log average of the AWF stain (23) was 260,000. The log average of the other five stains ranged between 160,000 and 240,000.

North (17) proposed the use of aniline oil–methylene blue stain for counting bacteria in dried milk and dried eggs. On 33 samples of dried milk he obtained an average of 302.5 million per gram, using his stain as compared with 82.7 million with the plain methylene blue stain. Levowitz and Weber (13) proposed a single solution stain referred to as the L-W stain. In a comparison of the aniline oil–methylene blue stain with the L-W stain, the American Dry Milk Institute (1) reported an average of 81.8 million for the former stain and 85.8 for the latter stain. In this study, 30 laboratories analyzed eight replicate samples with results in duplicate. In a similar study involving 24 laboratories, the United States Department of Agriculture (25) reported an average of 82.9 million for the aniline oil–methylene blue stain and 84.1 million for the L-W stain on samples of NDM.

Mantel and Robertson (14) made a cooperative study of six different stains on samples of raw and pasteurized milk and cream. These authors comment as follows: "Erratic counts were not confined to any particular milk stain or participating laboratory. Unusual counts could not be omitted from the comparison because there was no consistent way to identify them or to distinguish between them and many less erratic ones which were not completely out of line."

In this collaborative study all the smears were prepared in one laboratory and submitted to the others for staining and counting.

In a similar study, the ADMI (1) reported that the over-all average interlaboratory coefficient of variation was 183% for the North Aniline Oil–Methylene Blue stain, and 135% for the L-W stain. This study concludes: "This lack of agreement between laboratories indicates the necessity of further standardization of the Direct Microscopic Clump count method before it can be used on an industry-wide scale for specification purposes."

Levowitz (12), in a critical analysis of the Direct Microscopic Count, concluded as follows: "Official control administrators are ill advised to assume that the Standard Methods Direct Microscopic Count or any of the modifications proposed to this date can take the place of the Standards Methods agar plate count in the examination of raw milk for pasteurization." In a recent report to the National Conference on Interstate Milk Shipment (4), the standing laboratory committee recommended that the Direct Microscopic Count be deleted as an alternative method for the examination of raw milk intended for interstate shipment. This committee recommended that only the Standard Plate Count or a simplified method for viable counts be used.

In a report of a collaborative study on the DMC of nonfat dry milk, the USDA (25) concluded that the degree of reproducibility shows a direct relationship to the amount of special training and experience of the technicians and that even good technicians must spend several hours per day under immediate supervision for at least a month before their results can be considered reliable. On a selected group of four laboratories analyzing 100 samples, the interlaboratory coefficient of variation for all 24 laboratories averages 75.0%, using North's stain.

Black and Myers (5) presented a discussion of some of the errors in laboratory technique responsible for variations in the DMC of milk beyond those inherent in the method. They indicated that it should be recognized that the inherent error of the method is large, especially when applied to milk of high sanitary quality. They reported coefficients of variation ranging from 33 to 56% in the DMC obtained by individual workers in the same laboratory.

Morgan *et al.* (16) described a sequential procedure for grading raw milk by the DMC. Based upon their study, they concluded that the milk producer could not permit the count of his milk to exceed 108,000 DMC, to assure compliance 95% of the time with a legal limit of 200,000. This would correspond to an intralaboratory coefficient of variation of 43%.

Since nonfat dry milk is made from skimmilk obtained by separating whole milk, the effect of centrifugal force on bacterial cells of the milk could have a bearing on the relationship between the DMC of the raw milk and the DMC of the nonfat dry milk.

Leete (10), in a study of the viable count of 100 samples of whole milk, cream, and skimmilk, reported the removal of 23.2% of the viable bacteria by the separator.

Simonart *et al.*, in a series of papers published in Belgium (20-22), reported on the removal of bacteria from whole milk and skimmilk by centrifugation.

At a centrifugal force of 10,000 × G, spore formers are removed nearly quantitatively. *S. lactis* and *Staphylococcus aureus* are removed in greater proportion than *Escherichia coli, Proteus vulgaris,* or *Pseudomonas fluorescens.* These same investigators, using a centrifuge rated at 9,100 × G, with a capacity of about 7,000 pounds per hour, reported that between 86.3 to 97.6% of the bacteria originally present in the milk were removed. About 60 tests were made. Arph (2), using a centrifuge similar to that used by Simonart, reported that 97 to 99% of the spores and 76.2 to 96.6% of the bacterial cells were removed from skimmilk, which was a substantially greater removal than that obtained from whole milk.

Lembke (11), using a centrifuge with a capacity of 8,000 lb. per hour and a centrifugal force of 16,000 × G, reduced the viable bacteria count of raw milk by 77 to 98%.

From this review of the literature it would appear that the factors affecting the DMC of NDM could be dependent upon: (1) The amount of heat treatment given the milk. (2) The flora of bacteria present in the raw milk. (3) The kind of stain employed. (4) The kind and amount of centrifugal force applied during the separation of the whole milk.

Since the literature indicates that there is a wide variation in the results obtained on collaborative studies of the DMC, it is evident that the training and experience of the microscopist is also an important factor affecting the DMC obtained on any given sample of nonfat dry milk.

It is the purpose of this report to investigate some of the factors which may affect the DMC of NDM under commercial conditions. A knowledge of all of the factors involved is important in establishing product specifications and in determining compliance of a given sample to those specifications.

EXPERIMENTAL PROCEDURES

The milk used in this study was commercial milk obtained at various seasons of the year. The data reported cover the time from June, 1956, to March, 1959. Samples were taken from the raw milk and milk products resulting from it in either of two ways. Most samples were taken from bulk storage tanks of mixed raw milk containing 7,500 gal. Where line samples were taken at various points during processing, the samples were taken at 15-min. intervals for 1 hr., kept in an ice bath, and the four samples composited. The results obtained on three to five of these composite samples were averaged to obtain a value for a day's run, or lot. Samples of nonfat dry milk were taken at appropriate times when line samples were taken from the liquid milk products. Slides were made and stained promptly after the collection of the samples.

The method followed for the Direct Microscopic Clump Count (DMC) is described in Standard Methods for the Examination of Dairy Products (23) with few exceptions. These were that the stain used was the single solution stain described by Levowitz and Weber (13) and that ten fields were always counted regardless of the level of the DMC. A binocular microscope with a factor of 550,000 was used. The microscope lamp was rated at 100 watt. The DMC is reported on the per-milliliter basis on the liquid products, the condensed

milk being diluted with water to 10% total solids, but the DMC is reported on the per-gram basis for NDM.

RESULTS AND DISCUSSION

Relation between DMC of skimmilk and DMC of NDM. The principal basis for proposing the use of the DMC for grading NDM is to reveal the bacterial population of the raw milk from which the NDM was made. Accordingly, in the summer and fall of 1956, and the winter of 1957, a comparison was made between the DMC of the raw skimmilk and the DMC of the NDM (high-heat type). These results are presented in Table 1. In July, the DMC per gram of the NDM ranged from 1.9 to 23.7 times the DMC per milliliter of the skimmilk.

TABLE 1
Relation between the DMC of the raw skimmilk and the DMC of the nonfat dry milk made from it

Date	No. of days	DMC of skimmilk (millions/ml)	DMC of NDM (millions/g)	Times increase in count	Coefficient of correlation
July, 1956	10	27.7	164.0	5.9	+0.66
October, 1956	17	18.3	190.0	10.4	+0.53
January, 1957	17	2.6	53.5	20.2	+0.53

For example, on one day the DMC of the skimmilk was 24 million, whereas the DMC of the NDM made from it was 46 million. On another day, the DMC of the skimmilk was eight million and the DMC of the NDM made from it was 190 million. In October on one day, the DMC of the skimmilk was 15 million and the corresponding DMC of the NDM was 70 million (a 4.6 times increase in count). On another day in October, the DMC of the skimmilk was nine million and the DMC of the NDM was 200 million (a 22.2 times increase in count). The variation was greater in January, where on one day the DMC of the skimmilk was two million and the DMC of the NDM was 13 million (a 6.5 times increase in count). On another day, the DMC of the skimmilk was one million and the DMC of the NDM was 71 million (a 71 times increase in count). The mean increase in count was 9.1 times for the 44 days. The coefficient of correlation between the DMC of the skimmilk and the DMC of the NDM ranged from +0.53 to +0.66 for the three periods and indicates a general relationship between the two counts. The variation among days, however, is quite large, as shown by the range in the increase in count.

During the days that these studies were made, samples of the heated skimmilk, the condensed milk, and the NDM were taken at the start and end of each day's run. Standard plate counts were made on these samples and no evidence of bacterial contamination or multiplication of viable bacteria was found. Plant inspections by qualified personnel were made at regular intervals, to insure that plant sanitation was adequate and proper operating procedures were being followed.

Effect of separation on DMC of skimmilk. Since this study compared the skimmilk with the nonfat dry milk, a study was made of the relationship between the DMC of the whole milk and the DMC of the skimmilk. Fourteen trials, using three sizes of separators, are reported in Table 2. Two samples were taken for each of the 14 trials and the DMC was made in duplicate on each sample (a total of 56 observations). The mean DMC of the whole milk was 18.7 million, that of the skimmilk was 12.1 million, and that of the clarified skimmilk, 8.2 million. For 110 degrees of freedom, $t = 3.80$ and $P < 1\%$ for the whole milk × skimmilk. Similarly for skimmilk × clarified skimmilk, $t = 3.57$ and $P < 1\%$. The differences among the means are significant. In these trials, the individual skimmilk counts ranged from 41 to 112% of the whole milk counts and the mean was 65.8%. After separating the whole milk, the separators were cleaned and the skimmilk passed through again. A further reduction of 32.7% in the bacteria count occurred. Additional trials showed that each pass of the skimmilk through the separator resulted in a decrease in count even after six passes. The Model 192 De Laval Separator develops about 6,000 × g, whereas the Model 188 Clarifier develops about 9,000 × g. This latter force is in the range studied by the European workers (2, 11, 22).

TABLE 2
Effect of separation and clarification on the DMC of skimmilk

Separator	No. of trials	DMC		
		Whole milk	Skimmilk	Clarified Skimmilk
		(millions/ml)		
Farm Master (200 lb/hr)	6	12.5	8.4	6.5
De Laval No. 518 (800 lb/hr)	5	28.0	15.3	12.3
De Laval No. 192 (11,000 lb/hr)	3	15.6	12.7	6.0

Effect of breaking up of clumps on DMC. In May of 1957, a study was made of the relationship between the DMC of the raw milk as delivered in cans and the same milk after being received. Samples were taken from every can from about 120 producers. The milk was then weighed, pumped through a plate cooler to a storage tank, and held at 40° F. until all the milk from these producers was received (about 1 hr. elapsed time). A sample was then taken from the storage tank and compared with the producer count. This was done on 12 days. The coefficient of correlation between the mean DMC of the producer milk and the DMC of the storage tank milk for the 12 days was +0.912. The producer mean count was 9.4 million and the storage tank mean count 17.3 million. The smallest increase was 1.4 times, the largest increase 5.0 times, and the mean increase 1.85 times. Similar studies on Grade A milk have shown the same magnitude of increase in bacterial count made by the Standard Plate Method. To eliminate the possibility of bacterial contamination as a cause for the increase in count, swab tests were made of the milk contact surfaces in ad-

dition to visual inspection. The swab test results and the visual inspections were both satisfactory.

As the skimmilk is processed, further breaking up of clumps occurs. This is illustrated by the results in Table 3. There is first a decrease in count due to the effect of heat on the stainability of the bacterial cells. After each pumping of the skimmilk a slight increase in count results. Finally, there is a decrease in count due to the effect of drying.

TABLE 3
Progressive changes in the DMC of the skimmilk and condensed milk during the manufacture of nonfat dry milk
(Means of 36 line runs)

Source of sample	DMC (in millions per ml.)	Per cent of count of raw skimmilk
Raw skimmilk	22.4	100.0
Hot skimmilk (208° F/10 min.)	18.1	80.8
First effect of evaporator	18.7	83.5
Second effect of evaporator	19.0	84.8
Third effect of evaporator	19.8	88.4
Condensed milk to drier	21.4	95.5
Reconstituted nonfat dry milk	18.9	84.4

Effect of drying on DMC. The condensed milk generally has a higher DMC than the nonfat dry milk made from it. In a series of 32 comparisons, the mean DMC of the condensed milk was 11.6 million per milliliter, and the mean DMC of the corresponding NDM was 110 million per gram, or 94.3% of the DMC of the condensed skimmilk. These studies were made on nonfat dry milk having a whey protein nitrogen value of less than 1.0 mg/g (high heat type).

Effect of heat treatment of skim and storage after heating on DMC. There is a difference in the stainability of the bacterial cells due to the heat treatment given the skimmilk. Commercial trials were made comparing the relation between the DMC of the raw skimmilk and the DMC of the nonfat dry milk when the skimmilk was pasteurized at 160° F/15 sec., and when the skimmilk was given a heat treatment of 208° F/10 min. These trials, summarized in Table 4, showed that more stainable cells resulted from the higher heat treatment.

A series of experiments was made in the laboratory to study this effect of heating on the stainability of the cells. An additional factor—that of storage time—also was included. These results, shown in Table 5, confirm the previous observation that increased heat treatment of the skimmilk increased the stain-

TABLE 4
Effect of heat treatment of skimmilk on the relation between the DMC of the skimmilk and the DMC of the nonfat dry milk

Date	Heat treatment of skimmilk	No. of trials	DMC of raw skimmilk	DMC of NDM	Times increase in count
July, 1956	162° F/15 sec.	7	18.4	57.9	3.1
August, 1956	208° F/10 min.	10	13.8	82.0	5.9

TABLE 5
Effect of temperature of heating and storage time after heating on the DMC of skimmilk
(Means of 9 trials)

Heat treatment	DMC (in millions) after storage at 34° F.		
	(0 hr.)	*(24 hr.)*	*(48 hr.)*
Raw skim	14.1		....
150° F. for 5 min.	10.7 (75.9)[a]	5.5 (38.7)	4.0 (28.3)
180° F. for 5 min.	11.9 (84.6)	6.7 (47.2)	4.9 (34.6)
208° F. for 5 min.	12.7 (90.0)	7.6 (53.6)	4.4 (31.4)
245° F. for 15 min.	12.8 (90.6)	7.3 (51.6)	5.5 (38.8)

[a] Figures in parentheses are per cent of raw milk DMC's.

ability of the cells. These results also show that stainability decreases with increases in time of storage after heating. The lowest counts are obtained with the minimum temperature of pasteurization and the longest storage time thereafter.

While the results are not presented here, this same effect of decreasing stainability of the bacterial cells with increasing storage time applies to the reconstituted nonfat dry milk. Lower counts are obtained if the NDM is held cold for 12 or more hours after reconstituting and before preparing smears for counting.

A series of 18 samples of condensed skimmilk of about 40% total solids were tested for DMC when fresh, and after 4, 12, 24, and 48 hr. of storage at 35° F. This condensed milk was of the high heat type. The mean DMC fresh was 11.9 million; after 4 hr., 12.0 million; after 12 hr., 8.6 million; after 24 hr., 6.4 million; and after 48 hr., 4.3 million. While these values were obtained with the Levowitz-Weber stain, similar results were also obtained on the aniline oil–methylene blue stain and other stains tried.

Reducing the DMC of nonfat dry milk by varying processing methods. It is, therefore, apparent that the DMC of the NDM may be intentionally manipulated by varying at least two steps in processing. Table 6 summarizes the results obtained on four commercial runs of about 60,000 lb. of milk. A tank of whole raw milk was processed in two ways. Part of the tank was separated at 90° F., the skimmilk heated to 180° F. for 5 min., condensed to 40% solids, and immedi-

TABLE 6
Reducing the DMC of nonfat dry milk by varying two processing steps

Product and process	DMC (means of 4 trials)
Raw whole milk in storage tank 40° F.	14.1 million/ml
Skimmilk from above after separation 90° F.	11.8 million/ml
Skimmilk heated to 180° F/5 min. and condensed to 40% T.S.	7.6 million/ml
Nonfat dry milk from above	83.0 million/g
Same skimmilk as above, but clarified once after separation	8.5 million/ml
Same skimmilk heated to 180° F/5 min. and condensed to 40% T.S.	5.9 million/ml
Same condensed, held 19 hr. at 40° F.	3.1 million/ml
Nonfat dry milk	26.0 million/g

ately dried. The balance of the same whole milk was separated at 90° F. The skimmilk was clarified in a Model 188 De Laval Clarifier, heated to 180° F. for 5 min., condensed to 40% total solids, and cooled to 40° F. The cold condensed skimmilk was held 19 hr. and then dried. The first processing method yielded a nonfat dry milk with mean DMC of 83 million, while the second processing method yielded a mean DMC of 26 million. Thus, by clarifying the skimmilk and holding the condensed milk overnight, the DMC was reduced to one-third of its original value.

Influence of stain used. The data reported in the literature on the magnitude of counts obtained with various stains were obtained on raw or pasteurized milk. Since most of the cells observed in NDM are dead, stains which are satisfactory for living organisms may not be equally satisfactory for dead ones. Furthermore, in smears made from NDM, *S. lactis* types predominate, and these are rarely found in clumps. A study was made of several stains and the results are given in Table 7. Considering the various aspects of staining and counting, the Levowitz-Weber stain is preferred by this laboratory. The results, however, point out the need to specify the stain used when reporting results.

TABLE 7
Effect of stain used on the DMC of NDM
(Means of 32 samples)

Stain	DMC/g
Levowitz-Weber (13)	89.6
Aniline oil–methylene blue (23)	80.7
Broadhurst-Paley (7)	61.7
AWF (23)	60.4
Gray (8)	40.6
Polychrome MB (23)	40.0

Effect of flora present. Preliminary work has been done on more than a dozen organisms, to determine the effect of heat treatment and subsequent storage at 35° F. on their ability to stain. The results agree with published work, in that there is a wide variation in stainability secured with different organisms. Apparently, such factors as age of the cell, media, strain of organism used, type of stain, prior heat treatment of the skimmilk, and temperature of growth are important in determining quantitative reductions in stainable cells due to pasteurization and storage. Present results indicate small changes in numbers of stainable cells of *S. lactis, S. aureus,* and *Streptococcus thermophilus,* due to heat treatment or storage for 24 hr. Large reductions in numbers were observed in *Pseudomonas aeruginosa, P. fluorescens,* and *B. cereus.* The results obtained on other organisms were variable. More study is required to obtain a clearer understanding of the influence of the flora on the DMC of NDM.

Because the organisms in NDM are largely dead organisms, the microscopist is faced with an additional complication not encountered in grading raw milk. Organisms frequently appear as fragments, or ghosts, and it is sometimes difficult to decide what is an artifact and what is not.

It is not the purpose of this report, however, to evaluate the DMC of NDM from the standpoint of inherent errors in the method. The literature review includes the major work on this subject as applied to both raw milk and NDM. Work in this laboratory agrees with the data of Black and Myers (5) and Morgan *et al.* (16) on whole milk. Some smears made from samples of NDM, however, appear to have a greater variation in cell distribution than the distribution of organisms on slides made from skimmilk. In a series of studies on skimmilk and NDM, the coefficient of variation of clumps per field on the same smear was 21.7% for the skimmilk and 36.6% for the NDM. Calculations based on this difference indicated that twice as many fields should be counted when evaluating NDM as when evaluating skimmilk, to obtain the same degree of accuracy. Characteristics of the NDM may thus account for differences in collaborative studies reported in the literature (1, 25).

Evaluation of the DMC as a standard for grading NDM. The DMC is a useful tool for locating sources of contamination or bacterial growth within the drying plant. The DMC also serves as a broad index of the bacterial quality of the raw milk from which the NDM was made. However, the variations in the bacterial counts of the raw milk from day to day and from season to season are so large that a single bacterial standard can not be applied with satisfactory results to any unknown sample of NDM as a measure of plant contamination. The mere enumeration of dead bacteria is meaningless unless significance can be attached to their presence. Such significance, according to the data presented here, can be attached to the DMC of a sample of NDM only when its history is known.

A method which is to be used as a basis for a standard should be capable of a degree of reproducibility in conformity with the limits of the standard. As indicated by Morgan *et al.* (16) the working standard for a 200,000 DMC on raw milk is about one-half this value, to allow for errors in the method. Such lack of reproducibility is an inherent weakness of the DMC.

A third factor which detracts from the value of the DMC as a standard for grades of NDM is the excessive seasonal and geographic variations in the DMC of the raw milk. What is a meaningful standard in warm weather is not meaningful in cold weather.

Finally, any characteristic to be used as a basis for a standard must be incapable of subjection to deception. If a low DMC of NDM is interpreted as meaning that raw milk with a low bacterial population was used and was subsequently made into NDM by good commercial practices, no means of deception should be available to obscure this interpretation. Clarification of the skimmilk and storage of the condensed milk before drying, however, are conveniently done—and often necessary—yet these procedures have a profound effect on the DMC of the nonfat dry milk.

Because of the variations in the relationship between the DMC of the raw milk and the DMC of the NDM, and because of the many factors which may affect this relationship, the DMC of NDM does not appear to be a reliable method for evaluating the bacterial population of the raw milk from which the NDM was made.

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THE QUINHYDRONE ELECTRODE FOR pH DETERMINATIONS IN MILK AND DAIRY PRODUCTS[1]

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SUMMARY

Preliminary observations showed that the time interval between the addition of quinhydrone to milk and the potential reading had a highly significant influence upon the potential obtained. In spite of using a constant time interval, however, a difference between the pH obtained with a quinhydrone electrode and a glass electrode was found. The differences (pH glass electrode–pH quinhydrone electrode) averaged 0.08, with a standard deviation of 0.05 for the 60 whole milk samples included in the determinations.

Increasing the temperature in the milk-quinhydrone mixture caused increasing differences in the pH values determined by the glass electrode and the quinhydrone electrode in similar milk samples. Increasing amounts of serum solids also increased the differences.

When the fat content or the pH of the milk was increased, a decreasing difference between the value measured by the pH glass electrode and pH quinhydrone electrode was observed. Increasing temperature, serum solids, and pH in milk samples were shown to increase the pink color development in the milk-quinhydrone mixture as determined with a Hunter Color and Color Difference Meter, while addition of formaldehyde to the milk prevented the color formation. The results indicated that to obtain the best agreement between the two electrodes with milk samples a temperature of about 19° C. ought to be used, both in the calomel half-cell and in the milk-quinhydrone mixture. The differences observed between the pH of milk obtained with the glass electrode and the quinhydrone electrode probably can be explained by a reaction between quinone and proteins.

The determination of pH is a very useful aid in many types of routine dairy operations as well as in research work with dairy products.

During the 50-yr. history of pH determinations in milk and milk products, the use of the glass electrode and the quinhydrone electrode for pH determinations have seemed to be the most favorable. In fluid milk products, particularly if a continuous measurement of the pH is desired, the glass electrode is the most convenient. However, it has been shown by Dole (4, 5) that a difference in vapor pressures between the two sides of the glass membrane may cause great errors. This may be corrected for if the two vapor pressures are known. The use of the glass electrode for pH determinations in cheese may be questionable because the vapor pressure in the cheese is not known. Also, the high fat and the high protein content in the cheese may tend to tighten the pores in the glass membrane and thus cause erroneous results. For these reasons, the quinhydrone electrode generally has been considered better for pH determinations in cheese and has been used very extensively. The accuracy of the pH determinations in this manner, however, has seldom been discussed; but since the determination of pH

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in certain dairy products is most convenient by means of the quinhydrone electrode, its accuracy may have both theoretical and practical significance.

The first work on the use of the quinhydrone electrode in milk was reported by Lester (10). In ten milk samples she found that the quinhydrone electrode gave slightly lower values than the hydrogen electrode. Kolthoff (9) found for two analyses of milk a difference in pH values of −0.08 and −0.10 between the quinhydrone and the hydrogen electrode. In another sample of milk, in which the pH had been adjusted to 5.3 with acetic acid, he found a pH difference of +0.03 between the two types of electrodes. His results indicated that the pH of the sample may have some influence on the difference.

In this work, comparisons have been made of measurements of pH of milk by the quinhydrone and glass electrodes. It is hoped that the disclosure of factors responsible for differences observed may be useful in evaluating these two methods for measuring the pH of cheese and other dairy products.

The study of a comparison of the quinhydrone and glass electrodes for measuring pH of milk is divided into three phases for purposes of discussion: (a) the effect on pH values of milk of the amount of quinhydrone and time between adding quinhydrone and making the reading; (b) influence of certain factors on differences betwen pH values obtained with the two electrodes; (c) the red color development in milk-quinhydrone mixtures. Each phase will be considered separately.

METHODS

The equipment and chemicals generally used throughout this work have been:

(1) A gold electrode cast in a plastic rod used as a quinhydrone electrode.[3] (2) Leeds and Northrup Potentiometer No. 7655. (3) Leeds and Northrup Glass Electrode pH Meter No. 7664. (4) Calomel half-cell[4] with saturated KCl-solution.[5] (5) Quinhydrone.[6] (6) Mercury thermometer.

The use of specific equipment, chemicals, and methods will be discussed in connection with the individual experiments.

The glass electrode pH meter was standardized against a buffer at a pH about 4.0[7] and a buffer at a pH about 9.2.[8] The exact pH of the buffers varied with the temperature, so the pH meter was adjusted to both buffers by use of the standardizing and temperature dials.

The quinhydrone electrode was checked daily against a buffer solution at pH 7.0.[9] The accuracy of the thermometer was checked in ice water and in boiling water at a pressure of 750 mm. Hg.

Whole milk samples with as wide a range of pH values as possible were obtained by sampling milks of individual cows in the University of Wisconsin

[3] From Dairyland Food Laboratory, Waukesha, Wisconsin.
[4] Beckman Reference Electrode 270.
[5] Saturated KCl-solution for Beckman Calomel Electrodes.
[6] For chemical purposes. Eastman Organic Chemicals.
[7] Beckman Buffer 14044.
[8] Beckman Buffer 14049.
[9] Standard buffer solution. Cat. No. 11-505-230 from Fischer Scienetific Company.

dairy herds. The following breeds were represented: Holsteins, Guernsey, Ayrshire, and Jersey. The samples were usually kept overnight in a refrigerator at approximately 4° C. and tempered in a water bath to the desired temperature for each experiment before the pH determination was made. According to the work of Albrecht (1), the pH values obtained in refrigerated milk aged for 24 hr., therefore, were expected to be somewhat higher than pH values in freshly drawn milk. To eliminate the effect of possible increasing acidity while series of parallel determinations with the glass electrode and the quinhydrone electrode were being made, the pH in some samples selected at random was determined first by use of the glass electrode. In the other samples, the determinations were first made by the quinhydrone electrode. However, the time interval between two parallel determinations with the two electrodes was rarely longer than half an hour and the acid development was probably insignificant at the temperature of measurement. Milk samples were tempered for measurement in 5 to 10 min.

In every pH determination made by the quinhydrone electrode, the calomel half-cell was negative compared with the quinhydrone electrode. The electromotive force of the cell was, therefore, equal to the potential of the saturated calomel half-cell compared with the normal hydrogen electrode (E_{cal}) plus the potential observed. The normal potential of the quinone-hydroquinone system and E_{cal} were found for any temperature used, in a table given by Sommer (13).

The gold electrode was always rinsed in hot water between each single potential determination. Likewise, the glass electrode was washed with distilled water. Both electrodes were wiped off with soft paper. Between series of pH determinations the gold electrode was kept in a quinhydrone solution, while the glass electrode was kept in distilled water. The glass electrode was twice washed with distilled water at 50° C. to get rid of all fatty residue. To prevent increase in the temperature in the small milk sample in the plastic tube, the electrode and the tube were held in distilled water of a desired temperature about half a minute between the determinations.

The pH determinations with both electrodes were made in duplicate.

EXPERIMENTAL RESULTS

Effect on pH values of milk of the amount of quinhydrone and the time between quinhydrone addition and potential reading. The purpose of this series of experiments was to examine the necessity of using a constant amount of quinhydrone or a constant time interval after the quinhydrone addition before reading the potential. Quinhydrone was added in amounts of 5, 10, 20, and 40 mg. to 10-ml. samples of whole milk and the potential was observed 1.5, 5, and 10 min. after the milk and the quinhydrone had been mixed. The statistical treatment according to Walker and Lev (17), of the data observed in 24 different milk samples so treated, is shown in Table 1. The total number of analyses was 168.

As can be noted from Table 1, both the amount of quinhydrone added and the time interval between the time of adding the quinhydrone to the milk and the time of reading the potential had a significant effect on the pH value obtained. Also, there were significant interactions between the time interval and the amount,

TABLE 1

Variance analyses for the effect of the amount of quinhydrone added and the time interval between quinhydrone addition and potential reading on the pH values of whole milk samples

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Samples	23	1.378	0.059913	
Time	2	0.041	0.020500	39.27**
Amount	3	0.007	0.002333	3.09*
Time × amount	6	0.019	0.003167	9.93**
Time × sample	46	0.024	0.000522	1.64*
Amount × sample	69	0.052	0.000754	2.36**
Time × amount × sample	138	0.044	0.000319	

* Significant at the 5% level.
** Significant at the 1% level.

between the time interval and the individual sample, and between the amount of quinhydrone and the individual sample.

The average pH values for additions of 5, 10, 20, and 40 mg. of quinhydrone per 10 ml. of milk were 6.646, 6.647, 6.639, and 6.366, respectively. At time intervals 1.5, 5, and 10 min. after the quinhydrone addition, before reading the potential, the average pH values were 6.657, 6.640, and 6.628, respectively. Thus, increasing time interval between the quinhydrone addition and the observation of the potential caused decreasing pH values. The effect of the amount of quinhydrone seemed not to be significant when the amount was 10 mg. or less per 10 ml. milk.

Because of the high significance of the time interval, exactly 1.5 min. were used between the mixing of the milk and the quinhydrone and the determination of the potential in subsequent experiments. However, the amount of quinhydrone within the range of the amounts studied apparently had small practical significance. Therefore, exact weighing of the quinhydrone was not considered necessary. In succeeding experiments, attempts were made to keep the variation of the amount as small as possible by visual means and by using the same spatula for measuring quinhydrone.

To determine if there were any differences betwen the pH values obtained with the glass electrode pH meter and by use of the quinhydrone electrode, after standardizing the factors mentioned above, 60 different whole milk samples from individual cows were examined. The pH values of each sample were determined in duplicate by both methods. Following the recommendations of Van Slyke and Price (16), the temperature in the milk-quinhydrone mixture was adjusted as closely as possible to the temperature of that of the calomel half-cell. This varied between 22 and 25° C. according to the temperature in the laboratory. The distribution of the differences in the pH values obtained by the two methods (pH glass electrode–pH quinhydrone electrode) is shown in Figure 1. The average difference was 0.08 and the standard deviation 0.05. Statistical testing of the average showed a significant difference between the pH values obtained by the two methods.

Influence of some factors on the difference between the pH values of milk obtained with the glass electrode and the quinhydrone electrode. Other factors

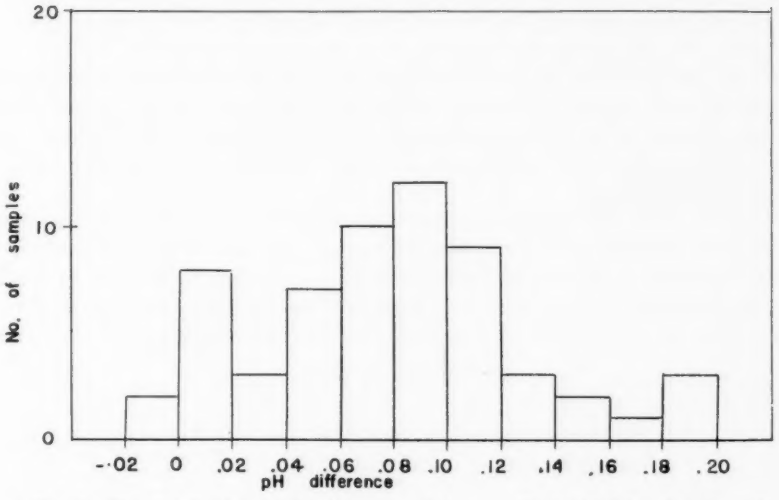

FIG. 1. The distribution of the differences between pH obtained by the glass electrode and by the quinhydrone electrode for 60 whole milk samples. (pH glass—pH quinhydrone).

which might be responsible for this difference in pH values as determined by the two different types of electrodes are: temperature of quinhydrone-milk mixture at time of reading, the acidity of the milk, the serum solids content, and the fat content of the samples.

It was the purpose of this next phase of study to investigate the influence of each of these factors on the pH values determined by the glass electrode and the quinhydrone electrode in similar milk samples.

Influence of temperature. Since temperature has an influence on the solubility product of salts, as well as on the dissociation constants of acids, bases, and salts, it undoubtedly has an effect on the pH value of milk. The commonly used procedure for the pH determination with the quinhydrone electrode specifies that the temperature in the calomel half-cell and the milk ought to be as close as possible. By means of a table it is then a simple matter to convert the potential readings to pH values. Døvle (6) has mentioned that there may also be potentials between the liquids used. This potential may change with the temperatures in the liquids, with the temperature difference betwen them, and with their chemical composition. Some reactions may also take place in the milk-quinhydrone mixture, which may cause the observed difference in pH values between the two electrodes to change according to changes in the temperature.

A relatively large sample of milk was used in the following experiments, to assure a constant temperature while the pH determinations were being made. Instead of the small plastic tube, a beaker containing about 100 ml. of milk-quinhydrone mixture was used. An agar bridge with saturated KCl solution connected the milk-quinhydrone mixture and the reference electrode through a saturated KCl solution. A preliminary series of experiments showed that there

was no significant difference in the pH values obtained in ten milk samples by use of the two different connections.

For the examination of the temperature effect, a water bath was used to give the desired temperature in the milk. When the temperature in the calomel half-cell was desired to be different from the room temperature, the half-cell was filled with saturated KCl solution of the same temperature for 10 min. before each potential reading.

Figure 2 shows the relationship observed between the temperature in the milk-quinhydrone mixture and the difference between the pH values obtained with the glass electrode and the quinhydrone electrode. A total of 80 observations of 20 different whole milk samples were made at approximately constant half-cell temperature ($23 \pm 1°$ C.). The regression curve can be expressed by the formula:

$$d = 20.026\, t - 0.55 \qquad (1)$$

in which

d = pH glass electrode − pH quinhydrone electrode

t = temperature in ° C. of the milk-quinhydrone mixture

The correlation coefficient, r_{dt}, was 0.953.

Figure 3 shows the difference (pH glass electrode—pH quinhydrone electrode) when temperatures of milk-quinhydrone mixture and the calomel half-cell were identical at each measurement, but when temperatures of measurements were varied from approximately 13 to 39° C. A total of 41 analyses were made. The

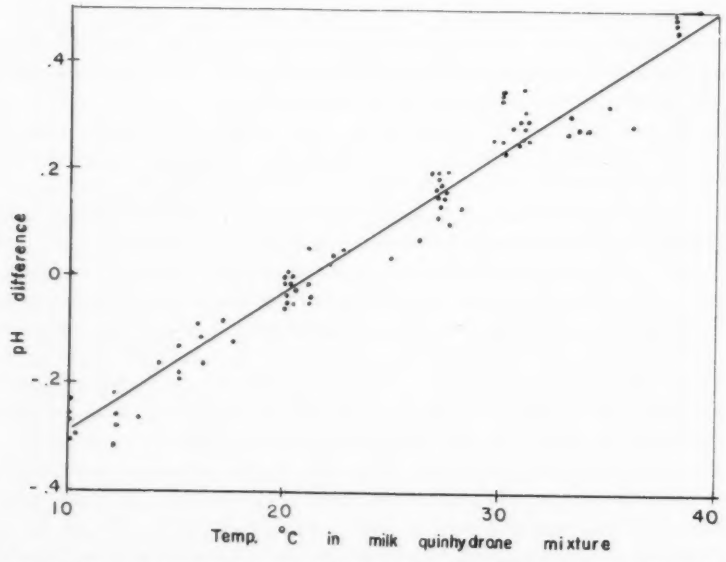

FIG. 2. The relationship between pH glass electrode—pH quinhydrone electrode and the temperature in the milk-quinhydrone mixture measured with the half-cell at constant temperature ($23 \pm 1°$ C.).

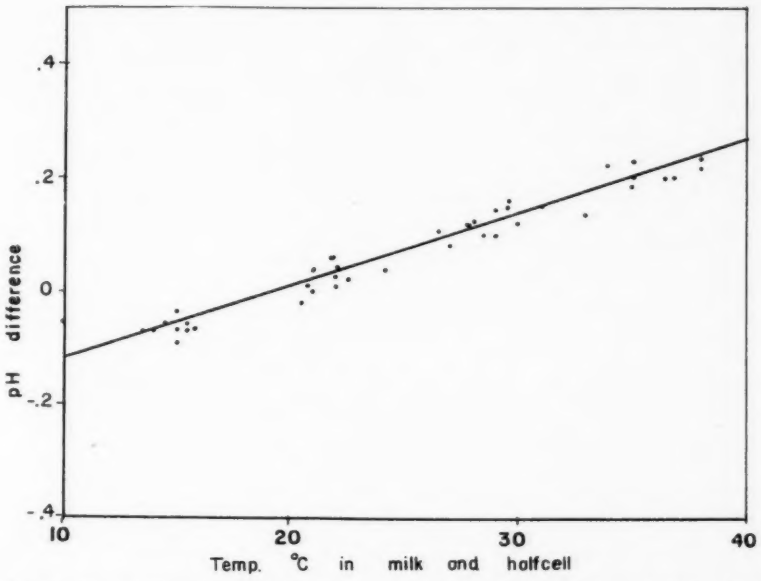

FIG. 3. The relationship between pH glass electrode—pH quinhydrone electrode when the temperatures in the milk-quinhydrone mixture and the calomel half-cell were the same at each temperature interval.

regression equation for the relationship, with t the temperature of the measurement, was:

$$d = 0.013\,t - 0.25 \qquad (2)$$

with r_{dt} 0.985.

The increase in the differences shown in Figures 2 and 3 at increasing temperature was highly significant. However, the temperature had a significantly less effect upon the difference when the temperatures in the two half-cells were equal than when the temperature in the milk-quinhydrone mixture varied and the calomel half-cell temperature was constant.

From Equation (2) it can be seen that with identical temperatures in the milk and the calomel half-cell (a commonly recommended procedure) the possibility for agreement was best at about 19° C.

When the temperature *(t)* in the calomel half-cell was varied and the temperature in the milk-quinhydrone mixture held constant (Figure 4), the following equation was found to express the relationship:

$$d = 0.210 - 0.010\,t \qquad (3)$$

This equation might explain the difference between Equations (1) and (2). The reason that the difference in this case was dependent on the temperature of the half-cell possibly may have been due to the influence of the temperature on the eventual liquid potential for the milk-quinhydrone/saturated KCl solution/

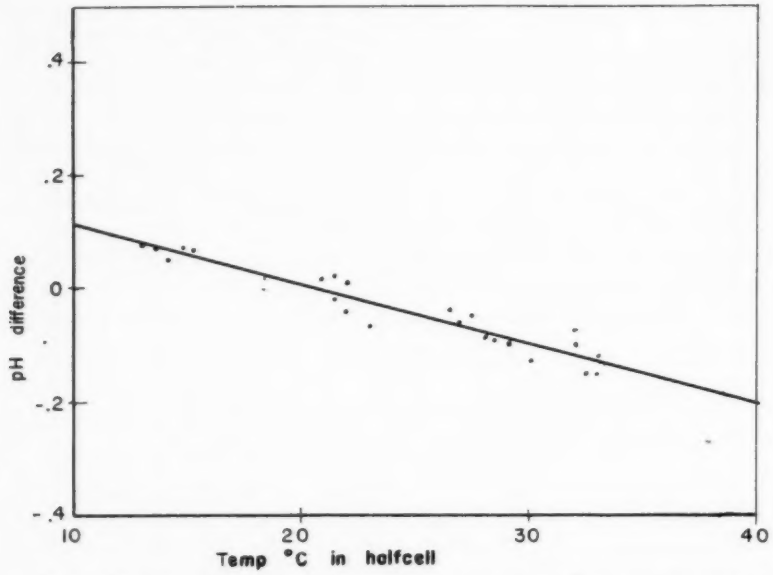

FIG. 4. The relationship between pH glass electrode—pH quinhydrone electrode and the temperature in the calomel half-cell when the milk-quinhydrone mixture was held at a constant temperature (20 ± 1° C.).

calomel system which may not have been fully included in the temperature factors used when calculating the pH values from the potential observed.

Influence of acidity. Varying amounts of lactic acid were added to pasteurized skimmilk to give a pH range from the normal down to 4.00, as measured by the glass electrode. The pH of these samples was also measured by means of the quinhydrone electrode. The differences (pH glass electrode–pH quinhydrone electrode) at the various pH levels in the skimmilk are shown in Figure 5. The correlation coefficient between the pH value determined by the glass electrode and the difference between this value and the pH value obtained with the quinhydrone electrode method was calculated to be:

$$0.935 \qquad (4)$$

Influence of serum solids content. Variation in the serum solids content of milk results in variation in the protein and lactose content and variations to a lesser degree in the content of salt and minor milk constituents.

Sørensen *et al.* (14) observed an error in the pH values determined with the quinhydrone electrode of −0.048 pH unit per mol NaCl when measured in .01 *N* HCl solution at different concentrations of NaCl. This NaCl concentration, however, far exceeded the content in milk, and the variation of NaCl due to variation in serum solids content has probably no significance. A possible protein error is also mentioned in works of Linderstrøm-Lang (11). Errors in pH measurements

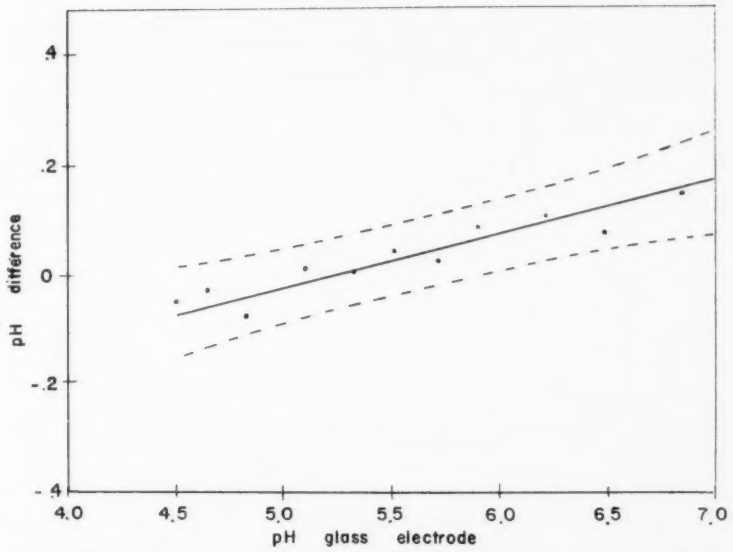

FIG. 5. The relationship betwen pH glass electrode—pH quinhydrone electrode and the pH obtained with the glass electrode in skimmilk samples acidified with lactic acid; pH values observed at 23° C.

- - - - - - - - Confidence belts for one analysis at 95% confidence limit.

of milk with the quinhydrone electrode due to other constituents in the serum solids were not found to be discussed in the literature. It is possible, however, that different salts may change the 50-50 distribution of quinone and hydroquinone, but a definite change in the pH value requires a marked shifting in the distribution.

High-quality nonfat dry milk solids were dissolved in distilled water to give 1, 2, 3, . . . 20 g. of serum solids per 100 g. of reconstituted skimmilk. The pH values were obtained in the samples with the glass electrode and the quinhydrone electrode. These observations have been plotted in Figure 6. The influence of the serum solids content *(C)* on the difference in the pH value *(d)* obtained with the two electrodes can be expressed by the equation:

$$d = 0.0076\,C - 0.005 \qquad (5)$$

with $r_{dC} = 0.897$. Thus, the difference was almost zero when the concentration was zero, and increased with increasing concentration. Increasing amount of serum solids caused also increasing acidity which, according to the results shown in Figure 5, should tend to decrease the difference in pH values. The increasing acidity, therefore, had possibly modified the difference obtained for the higher content of serum solids, and the effect of the serum solids at a constant pH value might be still greater than this experiment showed.

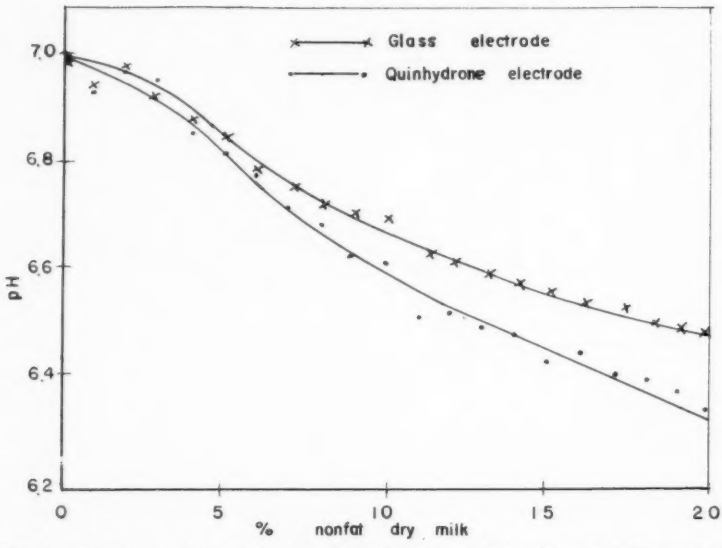

FIG. 6. pH values observed by the glass electrode and by the quinhydrone electrode at different amounts of serum solids in reconstituted nonfat dry milk. Temperature: 22.5 ± .5° C.

According to the work of Sørensen *et al.* (14), the salt content in the serum solids may be responsible for only a very small fraction of the regression coefficient in Equation (5). Possibly then, the protein content may have a definite influence on the difference between the pH values obtained with the two electrodes.

Influence of fat content. Knudsen and Sørensen (8) found that the pH determined with the quinhydrone electrode was lower in the cream than in the skimmilk from the same whole milk. Unmach (15) calculated that this difference could be explained if the solubility of quinone in milk fat were three times as great as the solubility of the hydroquinone. On that basis Døvle (6) calculated a correlation factor for pH equal to $-0.0035\ f$, where f was the fat content in the sample.

The difference in pH values between the cream and the skimmilk from the same whole milk, however, may be explained by a true difference in the hydrogen ion concentration. The negatively charged fat globules may tend to attract the positive hydrogen ions and thus cause actually lower pH in the cream than in the skimmilk.

As a means of determining the influence of the fat content on the pH as measured by the glass electrode and the quinhydrone electrode, samples of cream and skimmilk were mixed, so that the fat content ranged from 0 (skimmilk) to 32.7%. The pH of these mixtures was measured with both electrodes. The skimmilk and the cream were taken from mixed milk supplied by several herds to the University of Wisconsin manufacturing laboratory. The data obtained in these observations have been shown in Table 2.

TABLE 2

pH values obtained with the glass electrode and with the quinhydrone electrode in samples with different fat content. The temperature in the samples was 27° C.

Fat % (f)	pH glass (g)	pH quinhydrone (q)	Difference (g-q)	Fat % (f)	pH glass (g)	pH quinhydrone (q)	Difference (g-q)
0	6.79	6.53	0.26	18.0	6.77	6.63	0.14
1.6	6.76	6.54	0.22	19.6	6.74	6.64	0.10
3.3	6.76	6.56	0.20	21.3	6.78	6.65	0.13
4.9	6.75	6.58	0.17	22.9	6.76	6.66	0.10
6.5	6.77	6.58	0.19	24.5	6.80	6.64	0.16
8.2	6.73	6.58	0.15	26.2	6.76	6.68	0.08
9.8	6.76	6.59	0.17	27.8	6.82	6.68	0.14
11.4	6.73	6.59	0.14	29.4	6.78	6.69	0.09
13.1	6.77	6.63	0.14	31.1	6.79	6.69	0.10
14.7	6.78	6.62	0.16	32.7	6.78	6.70	0.08
16.4	6.75	6.61	0.14				

$r_{f \times d} = -.585^{**}$

** Significant at the 1% level.

The correlation coefficient between the per cent of fat and the difference (pH glass electrode—pH quinhydrone electrode) was significant and negative. This meant that the difference decreased when the fat content increased. This may be due to an effect of the fat content; but may also be explained with the effect of the serum solids as determined in an earlier experiment. When the fat increases, the serum solids content decreases, thus giving a positive relation between the amount of serum solids and the difference. The great difference between the pH determined by the glass electrode and the pH determined by the quinhydrone electrode is probably due to the high temperature (27° C.) of the samples. (See Figure 2.)

Red color development in the milk-quinhydrone mixture. Several investigators have observed that a red or pink color gradually develops in the milk after adding quinhydrone. The time after quinhydrone addition is considered to be of importance for the development of the color. Van Slyke and Price (16) point out that the potential should be determined in the mixture of quinhydrone and milk or cheese before the samples turn pink. A relationship between the drifting of the potential and the color is generally considered, but the nature of this has not been fully explained. If such a relationship exists, however, it may be logical to suppose a reaction between quinone or hydroquinone and one or more of the milk constituents in such a way that the same reaction results in both a red color and in a drifting of the potential.

Cooper and Nicholas (2) found that quinone reacted with several amino acids and other nitrogen-containing compounds. They noted that the amino acid–quinone complex usually had a reddish color. There has been little reference made in the literature to the work of Cooper and Nicholas in relation to the color development that occurs when pH determinations are made by use of the quinhydrone electrode in milk, milk products, and other protein-containing materials.

Fieser and Fieser (7) state that quinone readily combines with proteins, probably by addition reactions involving free amino or sulfhydryl groups. They

also emphasize that addition of NHR-groups has a marked lowering effect on the normal potential of the parent quinone.

A Hunter Color and Color Difference Meter[10] has been used to measure the development of the red color. This instrument gives three numbers, *L*, *a*, and *b* for each color measured. *L* is 100 at complete white color and 0 when the color is completely black. When the color is red, *a* is positive; it is 0 at grey color and negative when at green. A positive *b* means a yellow color; the number is negative when the color is blue and 0 at grey color. The numerical values of *a* and *b* increase with increasing intensity of the special color.

The milk samples were prepared as follows: To exactly 100 ml. of milk, tempered in a water bath to the desired temperature, a certain amount of quinhydrone was added. One minute after the addition, the mixture was strained through five layers of clean cheese cloth. This was done to remove quinhydrone particles which might cause errors in the readings if present at the time of the color determinations. The *L* value was then observed 4 min. after the quinhydrone addition, the *a* value observed 4.5 min. after, and the *b* value determined 5 min. after the addition of quinhydrone. The time intervals were exactly measured by use of a stop watch.

Calculation and testing of correlation coefficients were performed according to Snedecor (12).

Temperature, acidity, and serum solids content. The effect of the temperature, of the acidity, and also of different amounts of serum solids is shown in Tables 3, 4, and 5. The correlation coefficients show that all the three factors mentioned had effects on the color development. Increasing temperature caused a darker and reddish-grey color. With increasing pH values the redness and the yellowness increased, while the whiteness decreased. Increasing amount of serum solids also increased the redness and the yellowness. However, there was no significant correlation between the whiteness and the serum solids, probably because the reflection of light, measured as whiteness in the instrument used, is due to a certain amount of protein in the milk. Therefore, the Hunter Color and Color Difference

[10] From Henry A. Gardner Laboratory, Inc., Maryland.

TABLE 3

Whiteness, redness, and yellowness determined by the Hunter Color and Color Difference Meter in milk quinhydrone mixtures at different temperatures

Temperature (t) ° C.	Whiteness (*L*)	Redness (*a*)	Yellowness (*b*)
Blank (skimmilk)	82.3	−4.9	6.9
14.5	77.5	−3.0	6.8
21.5	74.7	4.7	6.8
22.5	73.4	6.0	6.4
23.0	75.5	4.2	6.3
31.0	73.1	7.5	4.7
32.0	70.8	9.0	4.8
35.0	69.6	10.0	4.1

$r^*_{t \times L} = -.936$; $r^*_{t \times a} = .935$; $r^*_{t \times b} = -.951$

* = Correlation coefficients.

TABLE 4

Whiteness, redness, and yellowness determined by the Hunter Color and Color Difference Meter in mixtures of reconstituted nonfat dry milk and quinhydrone at 19° C.

% Serum solids (s)	Whiteness (*L*)	Redness (*a*)	Yellowness (*b*)
2	47.5	−2.1	0.4
4	61.9	−2.5	1.5
6	67.8	−0.8	3.8
8	70.0	−0.4	5.0
10	71.3	0.2	5.4
12	71.5	1.1	5.9
14	71.4	1.6	6.2
16	72.7	0.8	6.4
18	66.5	5.0	6.7
20	70.4	2.5	6.5
	$r^*_{s \times L} = .646$; $r^*_{s \times a} = .901$; $r^*_{s \times b} = .901$		

* = Correlation coefficients.

TABLE 5

Whiteness, redness, and yellowness determined by the Hunter Color and Color Difference Meter in mixtures of quinhydrone and skimmilk samples acidified with lactic acid

pH determined with the glass electrode	Whiteness (*L*)	Redness (*a*)	Yellowness (*b*)
6.70	70.3	4.4	7.2
6.53	73.6	2.1	7.0
6.44	72.5	2.5	7.5
6.42	74.9	0.5	7.4
6.28	74.6	0.5	7.7
6.10	73.2	−0.2	7.9
5.80	75.6	−1.0	8.0
5.69	78.1	−2.6	8.1
5.59	78.0	−2.6	8.0
5.39	78.3	−3.4	8.2
	$r^*_{pH \times L} = -.926$; $r^*_{pH \times a} = .961$; $r^*_{pH \times b} = -.925$		

* = Correlation coefficients.

Meter showed less whiteness in the samples when the serum solid content was extremely small.

Generally, the factors which caused increasing difference between the pH values observed by the glass electrode and by the quinhydrone electrode also caused increasing redness in the milk quinhydrone mixture. There is, therefore, apparently a relationship between the color reaction and the pH value obtained by the quinhydrone electrode.

If the development of the reddish color in the milk-quinhydrone mixture might be due to some reactions between free reactive groups in the proteins and the quinone or the hydroquinone, it may be prevented by addition of formaldehyde to the milk before the quinhydrone addition. This possible effect was examined by determining the color development by the Hunter Color and Color Difference Meter after addition of quinhydrone. The quinhydrone was added to:

50 ml. whole milk + 10 ml. 40% formaldehyde

50 ml. whole milk + 10 ml. distilled water.

The relative amount of formaldehyde to amount of milk is the same as in the Walker formol titration.

TABLE 6

Effect of formaldehyde addition on the whiteness *(L)*, the redness *(a)*, and the yellowness *(b)* in whole milk samples mixed with quinhydrone

Sample No.	50 ml. milk + 10 ml. formaldehyde			50 ml. milk + 10 ml. distilled water		
	L	*a*	*b*	*L*	*a*	*b*
1	80.7	1.3	8.0	78.1	4.0	7.8
2	78.6	2.0	8.1	74.6	5.7	8.1
3	77.7	1.1	7.6	75.6	3.7	7.8
4	81.2	0.7	7.0	79.3	2.8	7.0
5	79.8	1.3	8.2	77.2	4.2	8.0
6	79.8	1.8	8.0	78.0	2.7	9.1
7	80.9	0.9	7.8	79.1	3.5	7.7
8	78.3	1.8	6.8	77.5	3.1	6.3
9	79.0	2.0	8.0	76.5	5.3	7.7
10	80.7	0.2	8.5	77.0	4.4	8.2
Average	79.7	1.3	7.8	77.3	3.9	7.8
			t value for *L*: 3.71**			
			t value for *a*: 7.13***			
			t value for *b*: .52			

** = Significant at the 1% level.
*** = Significant at the one per mille level.

The results for ten samples are shown in Table 6. The *t* values, together with the average color values, showed that the addition of formaldehyde caused a significant decrease in the development of the reddish color. The samples were also significantly whiter than the control samples. The formaldehyde, however, did not seem to have any influence on the yellowness in the samples. The effect of formaldehyde, however, need not only be due to the tying up of reactive groups in the proteins. The binding of basic groups will cause lower pH values in the milk and for that reason also prevent the color development.

DISCUSSION AND CONCLUSIONS

The experiments described showed a definite difference between the pH values obtained with the glass electrode and with the quinhydrone electrode in milk samples. While the amount of quinhydrone used in the determination was not of any practical significance as long as it was 10 mg. or less per 10 ml. of milk, the time interval between the addition of the quinhydrone to the milk and the determination of the potential had a highly significant influence upon the results. The influence of this factor was eliminated by using a constant time interval between the mixing of the quinhydrone with the milk sample and the determination of the pH in the sample with the quinhydrone electrode. This did not eliminate the difference between the pH readings observed on similar samples of milk by means of the glass electrode and the quinhydrone electrode. Though small, these differences were significant. However, they could be detected because of the smaller divisions on the longer scale of the Leeds and Northrup pH Meter used.

The results obtained seem to support the practice of using the same temperature in the calomel half-cell as in the milk-quinhydrone mixture. The temperature, however, had an apparently significant influence on the pH obtained, in

addition to the temperature effects which are corrected for by formulas commonly used. Thus, a temperature of approximately 19° C. in the milk-quinhydrone mixture seemed to give the best agreement betwen the pH obtained by the glass electrode and by the quinhydrone electrode. This may have importance for the practical use of quinhydrone electrodes because the temperature may often be as high as 27–30° C. in the laboratories. If used at such high temperature, the quinhydrone electrode will probably give pH values between 0.2 and 0.3 higher than those obtained with the glass electrode if used in whole milk.

The protein and the fat contents, which were shown to have some influence on the pH difference (pH glass electrode—pH quinhydrone electrode), undoubtedly have small significance in skim or whole milk because of the small variations in these factors. In cream, the pH obtained with the quinhydrone electrode will probably agree better with the pH obtained with the glass electrode than in whole milk samples at the same temperature. In dairy products with high protein content, the quinhydrone electrode will probably show lower pH values than the glass electrode.

However, for practical purposes in cheese factories, a knowledge of the absolutely true pH values in the cheese milk or in the cheese is not very important, if the method used for the pH determination gives reproducible and approximate results. The temperatures in the cheese milk or in the cheese at different stages of manufacture do not change significantly, and if the pH determinations are made on all samples at approximately 19° C., the quinhydrone electrode may be regarded as a reliable device for the control of the process.

The experiments described showed that increasing amount of serum solids, increasing temperature, and increasing pH caused increasing red color in the milk-quinhydrone mixture. These results may be explained by a possible reaction between quinone and proteins. Such reactions between free reactive groups in the proteins and the quinone may cause the following effects in the milk-quinhydrone mixture:

1. The proportion betwen the concentration of reductant and oxidant may be greater than one because some of the quinone molecules react with the proteins and thus leave the system. The effect on the potential of this shifting in the Red.-Ox. system may be relatively small, because there must be a rather large change in the one-to-one proportion of hydroquinone and quinone to cause a marked effect in the pH. Also, there probably may exist an equilibrium:

   $$\underset{\text{quinhydrone}}{\text{undissolved}} \rightleftarrows \underset{\text{quinhydrone}}{\text{dissolved}} \rightleftarrows \text{hydroquinone} \rightleftarrows \text{quinone}$$

   This will tend to maintain the equilibrium between hydroquinone and quinone when there is an excess of undissolved quinhydrone.

2. If quinone reacts with free groups in the proteins, a binding of basic groups is to be anticipated, and the acid character of the proteins may be more dominant and may cause an actual lower pH in the protein solution equal to the effect caused by formaldehyde addition. This view is supported by

the experiment which showed that addition of formaldehyde caused a decreasing development of the pink color. The number of uncharged groups increases as the acidity decreases, and this fact may explain why the red color decreased with decrease in the pH, as well as why the difference pH glass—pH quinhydrone decreased with decreasing pH. A probable shifting in the equilibriums, which undoubtedly exists, may explain why the time interval between the addition of the quinhydrone and the potential determination had a significant effect on the pH obtained with the quinhydrone electrode.

3. However, the statement of Fieser and Fieser (7), that the substitution of groups like NHR, NH_2, $N(CH_3)_2$, and $NHCOOH_3$ lower the red.-ox. potential of quinones, may have the greatest significance in connection with pH determinations in protein solutions by use of the quinhydrone electrode. The effect of possible substitutions on quinone is not found to be described in the literature, but the substitution of $NHCH_3$ to 1,4-naphthoquinone gives a lowering of the normal potential of −252 millivolts, according to data given by the authors just mentioned. A lowering of the normal potential in the Red.-Ox. system will cause a corresponding lowering in the pH value as determined by the quinhydrone electrode. If such great potential lowering effects also exist when proteins are substituted to quinone, which is possible, a substitution to only 1% of the quinone present in solution may cause a significant decrease in the apparent pH value.

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STUDIES ON PSYCHROPHILIC BACTERIA. I. DISTRIBUTION IN STORED COMMERCIAL DAIRY PRODUCTS [1,2]

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SUMMARY

Psychrophilic microorganisms capable of dominating the population in commercially pasteurized samples of milk, cream, chocolate drink, and cottage cheese after storage at 4° C. were isolated and characterized. Of 586 cultures, 70.6% were species of the genera *Pseudomonas*, 7.9% *Alcaligenes*, 9.2% *Achromobacter*, and 0.7% *Flavobacterium*; 10.8% were coliform bacteria and 0.8% yeasts. Distribution among the products, and between summer and winter samplings, was substantially uniform. Some factors underlying the discrepancies evident in the literature on the nature and distribution of the psychrophilic microflora of dairy products are discussed.

Investigators of the psychrophilic bacterial flora of dairy products have cited the occurrence of a great variety of genera including, on one or more occasions, *Pseudomonas*, *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Microbacterium*, *Corynebacterium*, *Alcaligenes*, *Achromobacter*, *Flavobacterium*, *Escherichia*, *Aerobacter*, *Proteus*, and *Bacillus*. The presence of still other genera has been implied, although the descriptions were incomplete. Notable discrepancies in the literature, both in regard to the experimental approach to the problem and to the results obtained, contribute to the difficulty of defining the psychrophilic flora and the significance of specific genera in spoilage of dairy products during refrigerated storage.

The present paper deals with the taxonomic groups of psychrophilic microorganisms isolated under strictly controlled and specified conditions from commercially pasteurized dairy products, and the relative frequencies of occurrence of these groups. An attempt is made to analyze the reasons underlying the lack of agreement among the several investigators.

MATERIALS AND METHODS

During one summer and the following winter the products of nine dairy manufacturing plants were sampled on a semiweekly schedule. Four hundred and seventy-three freshly packaged containers of whole milk, chocolate drink, coffee cream, whipping cream, and cottage cheese were transported to the laboratory under refrigeration. To permit selective development of the psychrophilic

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[2] Taken from data submitted to the graduate faculty of the University of Minnesota by W. D. Schultze in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Journal Series Paper No. 4116, Minnesota Agricultural Experiment Station.

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microflora, the samples were stored at 4° C. for seven days (except for cottage cheese stored for 14 days). Few samples showed obvious deterioration at the end of storage. Of those that did, most were cottage cheese samples. No initially spoiled samples were included. At the end of the storage period, samples were plated on Long and Hammer (11) agar and incubated at 7° C. for ten days. This medium, developed for the isolation of *Pseudomonas putrefaciens*, was chosen to enhance the recovery of this and possibly other fastidious species of *Pseudomonas*. We have observed no instance in which an organism culturable on plate count agar (Difco) failed to grow on this medium.

Portions of colonies representative of each colonial type appearing in the highest dilutions were subcultured and examined for gram reaction and purity. These isolates were coded as to source and maintained on Long and Hammer agar slants at 7° C.

Taxonomic studies were carried to the genus level on all isolates, employing observations of the following properties on cultures grown at 25° C.: gram staining reaction, cellular morphology, motility, flagellation (10), characteristics of growth in broth culture, production of diffusible fluorescent pigments (12), action on litmus milk, reduction of nitrate, lipolytic action on butteroil medium (5), hydrogen sulfide production, oxidase reaction (4), hydrolysis of casein, gelatin, and starch, and fermentation of glucose and lactose. Except as indicated by specific references above, the methods used were those recommended in the *Manual of Microbiological Methods* (13).

RESULTS

A total of 680 psychrophilic cultures were secured and studied in detail. Because of the danger of missing bacterial types inherent in selection solely by colony morphology, an effort was made to err on the side of inclusiveness. Consequently, biochemical studies proved that in many instances two or more isolates from a sample were identical. Such replicates were discarded. The remaining 586 cultures thus represented the microorganisms which had dominated the flora of samples after storage at 4° C.

All isolates, except for a few yeasts, were gram-negative, nonspore-forming bacilli, and belonged to one of the following five groups: the genera *Pseudomonas*, *Alcaligenes*, *Achromobacter*, and *Flavobacterium*, and the coliform group. Identification was based on Bergey's *Manual of Determinative Bacteriology*, 7th ed. (3). The use of this edition, published during the course of the studies, did not necessitate reclassification of any of the cultures. Taxonomic studies of psychrophiles based on editions of Bergey's *Manual* earlier than the fifth are, however, difficult to compare with contemporary reports.

In Table 1 is shown the frequency distribution of the psychrophilic groups obtained from each of the four dairy products. Species of the genus *Pseudomonas* were by far the most numerous psychrophiles encountered. The majority of these produced diffusible fluorescent pigments on asparagine medium. The other bacterial groups comprised only minor proportions of the isolates from any one product. The relatively high proportion of psychrophilic coliform

TABLE 1
Frequency distribution of psychrophilic groups as found in each dairy product

Group	Milk	Chocolate drink	Cream	Cottage cheese	All products	
	(%)				(No.)	(%)
Pseudomonas						
Fluorescent	45.1	39.3	39.5	64.8	270	46.1
Total	70.1	73.8	62.8	84.8	414	70.6
Alcaligenes	9.7	7.2	10.7	0.8	46	7.9
Achromobacter	9.7	8.3	9.9	8.0	54	9.2
Flavobacterium	1.6	0.0	0.8	0.0	4	0.7
Coliform	8.9	10.7	15.0	4.0	63	10.8
Yeast	0.0	0.0	0.8	2.4	5	0.8
Total isolates	124	84	253	125	586	

bacteria, 10.8% of the total cultures, was quite surprising in view of the paucity of reports of their occurrence in a psychrophilic microflora. The results of studies of these cultures will be published separately.

There was a striking similarity in the microbial flora of the products other than cottage cheese. The low incidence of species of *Alcaligenes* and of coliform bacteria in cottage cheese was outstanding. Examination of the data segregated by seasons showed only minor differences between summer and winter floras.

DISCUSSION

The term psychrophile has gained general acceptance for describing microorganisms capable of growth at relatively low temperatures. Since species of microorganisms exhibit continuous variation in their response to temperature, rather than falling into mutually exclusive categories, this basis for segregating them is inherently pragmatic. Thus, it is not surprising that definitions of psychrophilic growth have been numerous and conflicting. The distinction has been based on optimum temperature for growth or on ability to multiply at a significant rate below some arbitrarily chosen temperature. To cite only a few extreme differences in viewpoint, Erdman and Thornton (6, 7) and Thomé and Ljunggren (15) made use of 10.5 and 10° C., respectively, to isolate psychrophilic bacteria from milk and cream; whereas, Berry and Magoon (2) reserved the term psychrophile for organisms able to grow most rapidly at 0° C. or below, and admitted that none such had been found.

The conditions prescribed for culture isolation in the present study reflect the definition of psychrophiles considered by the authors to be most useful; namely, the ability to multiply sufficiently rapidly to become a significant contributor to the microflora at a given low temperature. Definitions based on optimum temperature are impractical because of the labor involved in determining the optimum temperature of a large number of cultures and the lack of data indicating the relationship between optimum temperature and the actual ability and rapidity of growth at refrigeration temperatures. For example, examination of the ability of certain of our cultures to grow at elevated temperatures has shown that most have an optimum temperature for growth in the range 25–30° C. and will grow even at 37° C. Indeed, consideration of optimum

temperature may be highly misleading, as indicated most recently by the report of Greene (8) that the influence of temperature on growth rate can be markedly altered by a change in the previous temperature history of the culture.

On the other hand, growth below some appropriate limiting temperature bears the closest possible relation to the practical methods for selectively isolating psychrophilic bacteria. Since the significance of psychrophiles is greatest in the field of food preservation by refrigeration, 4° C. was chosen for our isolations as being typical of good cold storage conditions in the dairy industry. The use of 10° C. seems too high for proper selectivity, and we believe this to be partly responsible for the reports of a large number of genera among psychrophilic floras. Lactic streptococci and enterococci, for example, may be isolated at 10° C., but their activity at this temperature and below is so restricted that they could scarcely be considered psychrophiles of importance in dairy products.

Rate of growth at the temperature of isolation is another important consideration which has not been consistently controlled in some previous studies. Erdman and Thornton (6, 7) isolated their psychrophilic cultures from platings of fresh milk and cream, and Thomas and Chandra Sekhar (14) held samples of milk for 24 hr. at atmospheric shade temperature before examination. The likelihood that isolations of psychrophiles made without a preliminary period of low-temperature storage would uncover a variety of essentially insignificant organisms may be inferred from the findings of Alexander and Higginbottom (1). In samples of pasteurized milk they found an initial psychrophilic microflora consisting chiefly of species of *Microbacterium*, accompanied by those of *Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Micrococcus, Bacillus*, and a few yeasts. After 10 wk. of storage at 6° C., the dominant flora had become restricted largely to gram-negative bacilli and yeasts. The results of Thomé and Ljunggren (15) show the relevance of this precaution with respect to the genus *Micrococcus*. Whereas micrococci comprised 86% of their cultures isolated from unstored pasteurized milk, they amounted to only 17% of the isolates after storage at 5° C.

A further difficulty in defining psychrophilic genera involves strictly taxonomic considerations. The inconvenience of determining the flagellar arrangement of a large number of cultures is granted, as well as some doubts about the wisdom of ascribing it major importance in taxonomy. Yet one can not classify the gram-negative bacilli according to the system presently in use without this information. Too often flagellation has been ignored in studies of psychrophiles (1, 6, 7, 9, 14, 15). Our experience suggests that this would lead, for example, to the misclassification of many atypical coliform bacteria. Other taxonomic criteria relevant to the gram-negative bacilli are rarely specific for one genus, and rarely without exceptions within a genus. Thus, if flagellation be excluded, it would be entirely possible to classify a gram-negative, asporogenous bacillus which produced no change in litmus milk or in carbohydrate broth and no fluorescent pigment in any of the three genera *Pseudomonas, Achromobacter,* or *Alcaligenes.*

It is recognized that our conditions for characterizing an organism as a psychrophile may not be entirely applicable for use with other food products, particularly those which, due to the nature of their composition and processing, normally are stored under refrigeration in commercial channels for longer periods. Under such conditions, genera other than those found in the present study undoubtedly assume importance. Whatever the product, it is felt that the conditions for isolation should reflect a close similarity to acceptable practices of low-temperature storage of the product or products concerned.

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STUDIES ON PSYCHROPHILIC BACTERIA. II. PSYCHROPHILIC COLIFORM BACTERIA IN STORED COMMERCIAL DAIRY PRODUCTS [1,2]

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SUMMARY

Coliform bacteria had previously been isolated as the dominant psychrophilic types from numerous samples of dairy products after storage at 4° C. for 1 wk. The 61 cultures were characterized according to their biochemical activities and, where possible, classified by species. As a group they showed tendencies toward more marked proteolytic activity and less gas production from lactose than would be expected of coliforms. The majority comprised a homogeneous subgroup which resembled *Aerobacter cloacae* most closely, but did not correspond in detail to any published description. They were distinguishable chiefly by a slight, delayed, and anaerogenous fermentation of lactose, an alkaline proteolytic reaction in litmus milk, a rapid hydrolysis of gelatin, inability to utilize D-tartrate, and the lack of arginine decarboxylase.

The ability of coliform bacteria to grow over a wide temperature range is generally recognized, but there is little published information as to the minimum temperature for growth of these microorganisms. Bergey's *Manual of Determinative Bacteriology*, 7th ed. (2), referred to growth at 10° C.; Foster *et al.* (5) stated that coliforms can grow slowly at refrigeration temperatures (3–5° C.). A practical minimum temperature of 10° C. was determined by Barber (1), with an average generation time at that temperature of about 865 min. Greene and Jezeski (8) isolated a strain of *Aerobacter aerogenes* from creamery water supplies, which was capable of attaining a population level of 10^6 per milliliter in ten days of incubation at 0° C. In some unpublished work by Thomas and Elson, as reported by Thomas (15), it was found that most raw milk samples held at refrigeration temperature contained psychrophilic coliform bacteria recoverable on violet red bile agar plates incubated at 3–5° C. for seven days. Of three cultures, including two species of *Aerobacter* isolated from raw milk stored at farm bulk-tank cooling temperatures, Marth and Frazier (10), found one culture able to grow in raw milk held for four days at 38° F.

Dahlberg (3, 4) was apparently among the first to report extensive growth of coliform bacteria in a refrigerated food, stating that in pasteurized milk

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[2] Taken from data submitted to the graduate faculty of the University of Minnesota by W. D. Schultze in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Journal Series Paper No. 4116, Minnesota Agricultural Experiment Station.

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stored at temperatures in the range 1.6–4.4° C. coliforms were able to multiply slowly and even to increase their proportion of the total microflora. Similarly, Olson *et al.* (11) demonstrated significant multiplication of coliform bacteria in commercially pasteurized milk during storage at 45° F., but in no instance did this group appear to dominate the flora and the authors discounted the role of these organisms in the deterioration of flavor of the samples. The opinion is commonly held that coliforms may occur sporadically in the psychrophilic microflora, but are relatively unimportant in food deterioration during refrigerated storage. Thus, Frazier (6) mentioned that the genus *Aerobacter* may contain psychrophilic species, but refrained from citing them as contributors to the spoilage of any refrigerated foods. Foster *et al.* (5) expressed much the same point of view.

In a previous paper (12), the authors reported the results of an investigation of the psychrophilic microflora of commercially pasteurized fluid dairy products and cottage cheese, in which it was found that 10.8% of the cultures were coliform bacteria. The existence of a relatively large proportion of coliform bacteria in a psychrophilic population was considered to be of sufficient interest to warrant further investigation, especially in view of the fact that the conditions of isolation gave assurance that each culture had multiplied sufficiently rapidly to attain a population of one million to two hundred and fifty million per milliliter, and to dominate the microflora of a sample, after 1 wk. of storage at 4° C. In the present paper, these psychrophilic coliform bacteria are characterized in greater detail and, where possible, classified according to species. The possible significance of such bacteria in the spoilage of dairy products is discussed.

MATERIALS AND METHODS

In addition to the methods employed in characterizing all microorganisms isolated in the survey, which have been described previously (12), those cultures tentatively identified as coliforms were examined as to characteristic growth on several commonly used differential media: Levine eosin methylene blue agar (BBL),[4] violet red bile agar (Difco),[5] Kligler's iron agar (Difco), and brilliant green bile broth, 2% (BBL). Utilization of citrate as sole carbon source was determined on slants of Simmons' citrate agar (Difco) after incubation at 25° C. for one day. The formation of indol in a 1% tryptone broth was tested by the Gore modification of the Ehrlich-Böhme technique on cultures incubated for two days at 25° C., and the methyl red reaction and O'Meara's test for acetylmethylcarbinol were performed after five and two days incubation, respectively, at 25° C., in the broth recommended for this purpose by Smith *et al.* (13). The methods used were those recommended in the *Manual of Microbiological Methods* (14). In addition, a group of 36 coliform isolates (see Group 5, Table 1) were submitted to the Enteric Bacteriology Unit of the Communicable Disease Center, Chamblee, Georgia, for supplemental characterization.

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TABLE 1

Characterization of subgroups of psychrophilic coliform isolates [a]

Group	No. of isolates	IMViC reaction	Litmus milk [b]	Nitrate red'n.[c]	Hydrolysis of:			Lactose [d]		Probable species
					Casein	Gelatin	Butteroil	Acid	Gas	
1	4	±+−+	Sl A	+	+	+	−	+1	−6	*Escherichia intermedia*
2	3	±+++	A, C, P	++	+	+	Sl	+1	Sl 6	Indeterminate
3	5	−−++	A, C, P	++	+	+	+	+1	+2	*Aerobacter cloacae*
4	13	−−++	A, ± C & P	++	Sl or −	−	−	+1	+2	*Aerobacter aerogenes*
5	36	−−++[e]	Alk P	++	+	+	+	Sl 6	−6	Indeterminate

[a] Reactions determined at 25° C.; [b] Sl = slight, A = acid, C = coagulation, P = proteolysis, Alk = alkaline; [c] + = reduction to nitrite, ++ = reduction beyond nitrite; [d] +1 = positive in one day, −6 = negative through six days, etc.; [e] −+−+ at 37° C.

RESULTS

Of the 586 psychrophilic cultures isolated and characterized, 61 were classified in the coliform group. They were gram-negative, asporogenous, straight rods, motile with peritrichous flagellation, produced acid and gas rapidly from glucose, reduced nitrate to nitrite and, in most instances, went beyond this stage. None produced prodigiosin or hydrolyzed urea.

The cultures could be segregated into five subgroups, as shown in Table 1. Of those cultures which produced the typical rapid acid fermentation of lactose, although with varying degrees of gas evolution, four resembled *Escherichia intermedia;* three isolates which were strongly proteolytic and had the IMViC pattern $\pm + + +$ remain unidentified; five were typical of *Aerobacter cloacae*, and 13 were strains of *A. aerogenes*. All the cultures except those in Subgroup Four showed proteolytic tendencies not usually attributed to coliform bacteria.

The isolates of Subgroup Five presented an unusually difficult taxonomic problem. They were atypical, in that they all produced a rapid alkaline proteolysis of litmus milk, their growth in lactose broth gave rise to only slight and delayed acid with no gas at 25° C., and no change at 37° C., and they liquefied gelatin in one day at 25° C. Their IMViC pattern was temperature-dependent, being commonly $- - + +$ at 25° C. and $- + - +$ at 37° C. Noteworthy from the standpoint of food spoilage was the rapid lipolytic activity on butteroil medium shown by all the cultures. This property was evident also in the cultures of *A. cloacae*. The biochemical characteristics of the atypical coliform cultures comprising Subgroup Five are shown in Table 2. They are given in terms of the reaction typical of the group and the percentage of the cultures giving a positive reaction; also indicated are the results of biochemical tests which gave conflicting results at 25 and 37° C.

DISCUSSION

Of the subgroups characterized in Table 1, the first four are noteworthy, chiefly in that they point to the existence of psychrophilic strains in the genus *Escherichia* and in the genus *Aerobacter*, though much more commonly in the latter. These organisms attacked lactose rapidly, and the resultant of their metabolic activities in litmus milk was acidic. Except for the 13 *A. aerogenes* strains, they hydrolyzed gelatin vigorously, but in general they fit the classical pattern of coliforms.

The cultures placed in Subgroup Five formed a homogeneous group which did not correspond in detail to any published description. They appeared to be aberrant forms of *A. cloacae*, distinguishable, in addition to the previously mentioned characteristics, by the inability to utilize D-tartrate, a somewhat less vigorous fermentation of all carbohydrates, and by the lack of agrinine decarboxylase. They also bore a strong resemblance to *Aerobacter liquefaciens*, which was isolated from butter and described by Grimes and Hennerty (9), although the latter species produced a typically gassy fermentation of lactose at 21° C. and an acid coagulum in litmus milk. The similarities were especially marked when our cultures were examined simultaneously with those of Grimes and

TABLE 2

Biochemical characteristics of atypical coliform isolates at 37° C.

Biochemical test	Reaction typical of the group	Cultures giving a positive reaction	Biochemical test	Reaction typical of the group	Cultures giving a positive reaction
Litmus milk—alkaline proteolysis	+	100	Lactose—37° C.	−	0
			—25° C.	x	100
Hydrogen sulfide production	−	0	Sucrose—acid	+	100
			—gas	x	86
Indol formation	−	0	Cellobiose—acid	Sl	61
			—gas	−	19
Methyl red—37° C.	+	97	Raffinose—acid	+	92
—25° C.	−	25	—gas	x	92
Voges-Proskauer—37° C.	−	0	Starch—acid	+	89
—25° C.	+	94	—gas	Sl	69
Motility	+	100	Salicin—acid	+	100
			—gas	x	83
Utilization of ammonium citrate	+	97	Rhamnose	−	100
Utilization of potassium citrate	±	50	Glycerol—acid	+	100
			—gas	x	89
Utilization of D-tartrate	−	0	Adonitol	−	0
Nitrate reduction	+	100	Dulcitol	−	0
Gelatin liquefaction	+	100	Sorbitol—acid	+	97
			—gas	Sl	86
Growth in KCN broth	+	97	Inositol	x	100
Urea hydrolysis	−	0	Malonate	−	0
Arabinose—acid	+	97	Mucate	−	0
—gas	−	33	Lysine decarboxylase	x	100
Xylose—acid	+	97	Ornithine decarboxylase	+	100
—gas	±	47	Arginine dearboxylase	−	0
Glucose—acid	+	100			
—gas	x	86			

− = negative; + = positive; Sl = slight; x = late positive; ± = highly variable.

Hennerty (9). The psychrophilic *Aerobacter* culture isolated from the water supply of a creamery by Greene (7) also was very similar to *A. liquefaciens*.

Many of the reports of types of coliform bacteria difficult to classify according to current schemes have been based on studies of dairy products. It is tempting to speculate that this might not be fortuitous, but rather that the distribution of physiological types occurring in the coliform flora of dairy products may differ from that found among coliforms from other sources. If it be true that atypical coliform bacteria, such as the cultures found in this study, or indeed any types of anaerogenous coliform bacteria, constitute a significant proportion of the coliform organisms in dairy products, they present a problem from the standpoint of routine laboratory identification. Because of their anaerogenous lactose fermentation such cultures do not fit the definition of coliform bacteria accepted for standard bacteriological examination of water or of milk, and they would be missed in a coliform test based upon gas production in liquid media. However, it is our experience that these organisms form typical colonies on the solid diagnostic media even at 37° C., despite the lack of detectable acid production in lactose broth at this temperature. It is interesting to note in this connection that Dahlberg (4) reported that at times he was able to detect coliforms on desoxycholate agar when the bacteria in the milk failed to produce gas in broth media.

Since our findings indicate that psychrophilic coliform bacteria may occur more frequently than has commonly been recognized, several reasons are suggested for the disparity of results. These microorganisms are superficially so different in biochemical activities from the classical pattern of coliform bacteria that their classification as such would be rather unlikely unless flagellation were determined. Also, if such organisms were isolated under temperature conditions not specific for psychrophiles, their rapid growth at 37° C. might well lead the investigator to ignore the possibility of their being capable also of relatively rapid growth at refrigeration temperatures.

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HIGH LEVELS OF GRASS SILAGE FOR MILK PRODUCTION WITH NO GRAIN, MEDIUM, AND HIGH GRAIN FEEDING. 1. INTAKE, MILK PRODUCTION, AND BODY WEIGHT CHANGES [1]

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SUMMARY

A three-lactation study with 18 Holstein and six Guernsey cows compared roughage intake, milk production, and weight changes on three levels of grain feeding (no grain, medium grain—1:6, and high grain—1:3) with maximum molasses-grass silage intake in addition to 5 lb. good alfalfa hay. Pasture was supplied during the summer. For each pound of grain fed, roughage dry matter intake decreased 0.23 lb. Average hay equivalent intake was 2.15 lb. over all groups. Average 245-day 4% FCM production for 12 cows with an estimated potential of 8,830 lb. was 6,490, 7,690, and 8,910 lb. on the three rations; for 12 cows with an estimated potential of 10,930 lb., production on the three rations was 8,080, 10,100, and 12,960 lb., respectively. There was no evidence of carry-over effects in the reversal phase or cumulative effects in the continuous phase. There was a close relationship between potential producing ability of the cows and response, measured as the ratio of extra milk produced per pound of grain fed, comparing production on high grain with that on no grain ($r = 0.88$).

Cows which had a 245-day potential of 8,900 lb. FCM gave 0.58 lb. milk per pound grain fed; whereas, those with a 245-day potential of 11,700 lb. FCM gave 1.35 lb. milk per pound grain fed. There was no evidence of diminishing returns in the high-potential cows, and relatively little for the lower-potential cows. Differences in rate of decline in production during the lactation were not apparent after the first 11 wk. of lactation, with little change after 6 wk. Though cows receiving no grain lost considerable weight from the first to the fourth month (−106 lb.), compared with the high-grain cows (+16 lb.), they gained more in the latter part of lactation (190 vs. 142 lb.); however, they still did not regain their precalving weight in 50 wk. High-grain feeding may be profitable for cows with the potential for high production, but not for cows with lower potential, when fed large amounts of good-quality molasses-grass silage.

With the increasing emphasis on grassland farming, the most desirable level of concentrate feeding becomes a more important factor. Many studies in the past were concerned, in one way or another, with this subject. In some studies top-quality irrigated alfalfa has been used; others, though excellently designed, were conducted over short periods of time, and in still others the emphasis was on use of hay. In the northeastern states particularly, and in other sections in lesser degree, the high percentage of rainy and high-humidity days prevents the making of consistently good-quality hay, especially if cut early for highest nutritive value. Under these conditions, the crop may be most effectively preserved as silage.

It was the purpose of this study to determine what might be expected from feeding all the good-quality, farm-grown roughage that the cows would eat, when

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most of it was in the form of grass silage, with varying amounts of concentrates. It was considered important that the study cover a period of time sufficient to determine whether cumulative effects would develop which would not be apparent in within-lactation studies, and also to determine whether carry-over effects between lactations could be measured.

EXPERIMENTAL PROCEDURE

The only controlled difference among ration groups was in rate of concentrate feeding. Cows on Ration O (no-grain) received no concentrate, on Ration M (medium-grain), a concentrate mixture at a rate of 1 lb. per 6 lb. 4% fat-corrected milk (hereafter referred to as FCM), and on Ration H (high-grain), concentrates at a rate of 1 lb. per 3 lb. FCM. Adjustments in grain feeding were made for each cow, based on her milk production during the previous week and the average butterfat test from two consecutive milkings during that week. If more than 18 lb. per day were required at the given rate, the excess was held and fed after the required amount dropped below 18 lb. The concentrates were specially mixed during the first year, but a commercial mixture was used for the remainder of the experiment.

Hay fed during the barn period was alfalfa hay of good to excellent quality and was limited to 5 lb. per cow at one feeding per day.

Grass silage was fed four times daily, at about 5:45 A.M., 8 A.M., 3:45 P.M., and 7 P.M., in amounts slightly larger than the cows would clean up; the uneaten feed was weighed out twice daily. The silage was first-cutting alfalfa-grass mixtures, cut in the early-bloom stage of the alfalfa and ensiled with about 80 lb. molasses per ton. One silo was reserved for this experiment and the silage was relatively uniform during a season. Due to winter killing of alfalfa, the silage was low in alfalfa the first year, with increasing proportions in each succeeding year.

A mineral supplement of equal parts salt and dicalcium phosphate was fed during the barn periods.

During the pasture season, hay was fed ad libitum, either in the barn or in a rack in a paddock. During the first summer, cows were on pasture at all times except at milking time; in subsequent summers, they were on pasture for three periods for a total of 8.5 hr. per day, with the remaining time in a dry lot with hay and water available. This schedule was used to extend the available pasture over a longer time, but resulted in less efficient use of the pasture (3). Most of the pastures were mixtures of Ladino clover with orchard grass or brome grass, with percentages of orchard grass increasing from year to year. Drought severely affected the pastures during this study, with total pasture days for 1953, 1954, and 1955 being 64, 152, and 146, respectively. During all 3 yr. there were periods when silage was fed to the cows to replace pasture. Individual records were not kept at this time.

Proximate analysis was carried out on samples of all feeds collected periodically, by methods of the Association of Official Agricultural Chemists (2). Total digestible nutrient (TDN) content of the feeds was estimated from chemical composition by the regression method of Schneider *et al.* (22, 23). These esti-

mated TDN values were substituted in the regression formula of Moore *et al.* (15), to obtain values comparable to Morrison's estimated net energy values (16); these will be referred to as calculated net energy values (CNE).

Since both cumulative and carry-over effects were to be studied, the experiment was divided into two phases, a continuous phase to measure accumulation of effects from lactation to lactation on the same ration, and a change-over or reversal phase to permit measurement of carry-over or residual effects between lactations, both covering three lactations.

The continuous phase (Phase C) consisted of a randomized block design with four groups of three Holstein cows; each cow received the same ration throughout the experiment. The change-over or reversal phase (Phase R) consisted of two balanced pairs of 3×3 Latin squares proposed by Cochran *et al.* (4), with two groups each of Holstein and Guernsey cows; cows were changed to the new ration after calving. Selection was on the basis of health, freedom from mastitis, and probability of completing a three-lactation study. Each group was as uniform as possible, with primary emphasis on the milk-producing potential estimated from mature equivalent FCM in at least one previous lactation. The most uniform groups with the largest numbers of records were assigned to the continuous phase. All cows were milked twice daily. Age correction was by Kendrick's factors (8), and calculation to a 4% fat-corrected milk basis was by use of Perkins' factors (18).

To evade the problem of differing effects of stage of gestation, production records were calculated on the first 245 days of the lactation, as well as on a 305-day basis. The analysis was based on the 245-day records.

Some cows did not complete the desired three lactations, due to disease or reproductive failure. Two cows were replaced after the first lactation, and two lactations were deleted due to mastitis. In the continuous phase (C), eight of 24 possible lactation values were missing. In spite of the large number of missing values, the most satisfactory method of using the remaining data was to estimate missing values; the randomized block formula and the iterative procedure (24) were used, with lactation and cow as the two classifications, ignoring group and treatment. In the change-over phase (R), Bartlett's dummy covariate method was used, with the modification that the regressions were calculated on the treatment by group interaction plus residual sums of squares and products; the two missing values were in the same square and on the same treatment. These calculated values were considered as expected values rather than merely steps in the statistical analysis (17), and were included in means presented here.

All cows were weighed on three successive days every 3 mo. and on one day at the end of each month between the quarterly weighings.

The chemical analysis of the feeds used, presented by years (Table 1), showed that the protein content of the silage increased each year, undoubtedly as a result of the higher legume content; crude fiber showed a progressive decline, possibly as a result of somewhat earlier cutting. These changes were reflected in a trend for both TDN and CNE values to increase.

TABLE 1
Chemical analysis, TDN, and CNE of feeds used

Year	Dry matter	Dry matter basis						
		C.P.[a]	E.E.	C.F.	Ash	N-F.E.	TDN	CNE
	(%)							*(therms per cwt.)*
Silage								
1952–53	27.0	10.6	4.8	38.7	6.6	39.3	56.9	44.6
1953–54	27.0	12.6	4.7	36.4	7.6	38.7	59.2	47.8
1954–55	22.8	13.6	3.7	31.5	8.6	42.6	60.4	49.5
1955–56	25.0	14.1	4.0	31.3	7.9	42.7	61.6	51.1
Hay								
1952–53	88.8	20.0	1.5	27.7	9.6	41.2	61.6	51.2
1953–54	93.1	17.6	2.4	27.6	7.4	45.1	62.1	51.9
1954–55	89.7	18.7	2.0	26.2	7.8	45.4	63.3	52.5
1955–56	84.7	21.2	2.1	26.0	9.5	41.2	63.2	53.4
Concentrates								
1952–56	86.6	20.1	4.6	7.7	6.4	61.2	79.5	76.1

[a] C.P. = crude protein; E.E. = ether extract; C.F. = crude fiber; N-F.E. = nitrogen-free extract; TDN = estimated total digestible nutrients (regression formulas of Schneider *et al.*, 22, 23); CNE = calculated net energy (regression formula of Moore *et al.*, 15).

Statistical procedures followed include analyses of variance and covariance as described by Snedecor (24) and by Federer (5).

RESULTS AND DISCUSSION

Effect of grain feeding on roughage consumption. The study of this relationship was made on the data from the barn periods. The average daily roughage and concentrate intake values by ration and group are presented (Table 2), to show the possible relationship of plane of nutrition to forage intake. For each pound of concentrate fed on Ration M, there was an average decline of 0.21 lb. forage dry matter; at the higher level, from Rations M to H, the decline was slightly higher, 0.26 lb., for an over-all average of 0.235 ± 0.012. This difference between the two steps, although not significant, was in the direction anticipated.

TABLE 2
Relationship between concentrate feeding and forage intake during the barn period of 245-day lactations by groups, in terms of pounds dry matter per day

Group	Concentrates			Forage			Change in forage intake per lb. concentrate (F/C[a])		
	O	M	H	O	M	H	M-O	H-M	H-O
C1H[b]	0	6.3	14.5	26.7	24.3	24.0	−.38	−.04	−.18[a]
C2H	0	6.7	15.2	23.7	24.7	20.9	+.15	−.45	−.18
C3H	0	7.0	14.5	26.0	23.7	21.7	−.33	−.27	−.30
C4H	0	7.0	11.9	27.3	24.2	21.3	−.44	−.59	−.51
R5H	0	5.2	11.8	24.9	23.2	23.6	−.33	+.06	−.11
R6H	0	5.2	8.3	27.4	25.4	22.7	−.38	−.87	−.57
R7G	0	5.4	11.3	22.4	22.8	22.4	+.07	−.06	−.01
R8G	0	4.5	8.5	18.7	18.9	18.1	+.04	−.20	−.07
Mean	0	5.9	12.0	24.7	23.4	21.8	−.21	−.26	−.23

[a] For example: Forage (H-O)/Concentrates (H-O) = (24.0 − 26.7)/(14.5 − 0) = −2.7/14.5 = −.18.
[b] C = continuous phase, R = change-over phase, H = Holstein, G = Guernsey.

Within each breed and phase, the groups with the highest potential production (Table 3) declined least in forage intake due to the grain feeding (P *ca.* 0.04). On the average, for each 1,000-lb. increase in production, this effect of grain feeding on forage intake decreased 0.17 ± 0.056 lb. This increased appetite of the higher potential cows may thus be a cause, rather than an effect, of the higher production of these cows. With the system of feeding used in this study, a ratio of grain to milk, the higher-producing cow tended to be fed at a lower plane of nutrition than the lower-producing cows. The relationship between change in plane of nutrition from Ration O to Ration H, determined as the

TABLE 3

Production of 4% fat-corrected milk per day for 245 days by ration and group (potential and experimental)

Group	Potential	FCM on ration			Comparisons			Ratio FCM Conc.
		O	M	H	M-O	H-M	H-O	H-O
C1H	47.9	33.5(2)[a]	40.0(1)	58.2(1)	6.5	18.1	24.7	1.40
C2H	47.9	30.4	43.6	54.1(2)	13.2	10.5	23.7	1.29
C3H	44.2	34.2	45.1	51.3	11.0	6.2	17.2	1.11
C4H	38.4	33.7(1)	36.1(1)	47.9	2.4	11.8	14.2	0.94
R5H	40.0	33.4	32.9	44.0	−0.5	11.1	10.6	0.76
R6H	36.5	29.1	31.9(2)	36.0	2.7	4.1	6.8	0.58
R7G	35.2	28.2	37.3	39.9	9.1	2.6	11.7	1.02
R8G	32.5	15.2[b]	22.6[c]	25.6	7.4	3.0	10.3	1.22
Mean—C groups	44.6	33.0(3)	41.2(2)	52.9(3)	8.3	11.7	19.9	
% of lower level					125	128	160	
% of higher level					80	78	62	
Mean—R groups	36.0	26.5	31.4(2)	36.4	4.7	5.2	9.9	
% of lower level					117	116	137	
% of higher level					84	86	73	
Mean—All groups	40.3	29.8(3)	36.3(4)	44.6(3)	6.5	8.3	14.8	

[a] Figures in parentheses indicate number of estimated values included in average.
[b] Includes one complete lactation (Cow 880) of 176 days only; mean figured as if 245 days.
[c] Includes one complete lactation (Cow 880) of 193 days only; mean figured as if 245 days.

fraction of estimated requirements actually consumed, was also compared with the change in forage intake. The relationship here was not as close as with potential (P *ca.* 0.09), and partial regression showed little effect of plane of nutrition after consideration of potential production.

By analysis of covariance, the among-rations regression of forage intake on grain feeding was −0.235 ± 0.012. Regressions for all sources of variation in the analysis of covariance were negative except among groups (0.81 ± 0.67), again indicating that those cows that had the appetite to eat more roughage

produced more milk, and this would lead to the feeding of more concentrates, a compounding effect. Even though not significant, it is suggestive. The over-all regression (all data) was -0.188 ± 0.076.

Because of the pronounced effect of concentrates on body condition, size of the animals was not adjusted for in the values above. Since the average body weight of the Ration O cows 60 days before calving at the beginning of the experiment was 1,277 lb., compared with 1,272 lb. for those on Ration H, any adjustment would have tended to introduce more bias than it would have corrected. However, when covariance adjustment for body weight was made, the among-ration regression was -0.347 but with no degrees of freedom for error (*cf.* -0.235 unadjusted).

These values for effect of concentrate feeding on forage intake are lower than reported elsewhere, where values from -0.4 to -0.8 have been cited, the lower values being associated with better quality forages and the higher values with poorer forages (20). When U.S. No. 2 alfalfa hay and corn silage were fed to cows with a 305-day potential of 6,900 lb. FCM, increasing grain feeding from a 1:4 rate to 1:3 caused a decrease in roughage dry matter intake of 1.11 lb. per pound grain dry matter fed. Going from a 1:3 rate to 1:2 caused an even larger decrease, 1.67 lb. (13).

Relationship between body size and roughage intake. On the usual basis of a simple proportional relationship between intake and size, the average forage dry matter intakes per hundredweight for Rations O, M, and H were 2.11, 1.88, and 1.68 lb., or in terms of 88% dry matter-hay-equivalent per hundredweight, were 2.40, 2.14, and 1.91 lb., respectively. The average of 2.15 lb. hay equivalent is above what would be called average forage intake (2.0 lb.), but lower than has been reported in other studies where the highest-quality forage was selected (6, 9, 10, 11).

As has been suggested by Legates *et al.* (11), the simple ratio tends to give a false picture of the relationship between size and intake. The true relationship is probably curvilinear, but for the relatively short range of sizes of cows in the experiment the linear regression is probably adequate and is somewhat more realistic than the simple ratio. On the total variation basis (individual observations) there was an increased intake of forage dry matter per hundredweight of 0.62 ± 0.27 lb., which is markedly below the above ratios. Calculation on a within-ration basis removed much of the confounding influence of differences in condition and showed an increase of 1.02 ± 0.25 lb. per hundredweight.

The average ratios of pounds forage dry matter intake to (1,000-lb. body weight)$^{.73}$, which is the same as adjusting to a 1,000-lb. body weight basis in proportion to $W_{lb.}^{.73}$, as suggested by Mather and Rimm (14), for rations O, M, and H, were 22.0, 19.9, and 18.1 lb. (25.0, 22.7, and 20.5 lb. hay equivalent).

Milk production. Fat-corrected milk production was summarized by groups and rations (Table 3), together with the potential production estimated from previous records and experimental production as percentages of each level, all on a daily basis. In terms of 245-day milk totals, the estimated potential production was 10,928 lb. for the groups in the continuous phase, and experimental

productions on Rations O, M, and H were 8,076, 10,097, and 12,957 lb., respectively; in the reversal groups, estimated potential was 8,829 lb. and experimental productions were 6,492, 7,688, and 8,910 lb., respectively. The increase in pounds FCM and percentage from O to M and from M to H were both greater in Phase C than in Phase R.

The possibility of cumulative effects in Phase C was evaluated by comparing the average change per cow from the first to third lactations; the increases for Rations O, M, and H were 5.0, 9.9, and 4.7 lb. milk daily. As the increase on H was less than on O, there seemed no indication of a cumulative effect. The interaction between ration and year was not significant [(24), p. 313].

The estimates of the carry-over effect in Phase R for Rations O, M, and H were +0.52, −0.16, and −0.36 lb. FCM daily (4). These were negligibly small ($F < 1$), and again the carry-over effect of Ration H was less than for Ration O.

This failure to find either cumulative effects of the same ration or carry-over effects from different rations was somewhat unexpected. Cochran *et al.* (4) found that when levels of feeding were changed during a lactation there were definite carry-over effects (levels of feeding were all roughage, 1:7, and 1:3.5). Furthermore, it has been commonly accepted that cows in high condition at the time of calving will produce more in the succeeding lactation than cows in moderate or thin flesh. However, Pratt (19) reported that Jersey cows on unlimited roughage, which received grain at 1 lb. per 3 lb. FCM, declined in mature equivalent FCM in successive lactations; whereas, cows receiving about 1 lb. per 4.6 lb. FCM increased in the second, third, and fourth lactations, equaling the higher grain cows at the second lactation. More recently, the report of Schmidt and Schultz (21) indicated that condition of cows at calving did not affect subsequent milk production.

Thus, the design itself, or differences in cumulative or carry-over effects, could not be considered as the explanation of the different response of the two phases. The producing capacity of the C cows was markedly higher than the R cows, whether compared on the basis of potential or on experimental production. Also, though the rate of grain feeding, as a ratio, was the same in all groups, the amounts of grain actually fed differed, as already shown (Table 2). If the difference in response was associated with level of potential production, the interaction of ration with group would be a gross measure of the effect. In Phase C it was significant (P *ca.* 0.01), but in Phase R, with a larger error term in the analysis of variance, the interaction was not significant.

For a more refined evaluation of the effect, the two phases were combined and divided on the basis of breed. Comparison of the production response on Ration H over Ration O with potential production showed the very high correlation of 0.95 ($P < 0.01$). The correlation between the increase in FCM and the actual grain fed on Ration H was also very high, 0.93 ($P < 0.01$). It is of particular interest that the correlation between potential production, based on previous lactations, and grain consumption during the experiment was about the same size, 0.91 ($P < 0.01$). Thus, the cows with higher potential could be considered as responding more to the first increments of grain after calving,

which in turn brought about the feeding of more grain with the system used. Coupled with this may be the fact already brought out, that high-potential cows did not decrease as much in roughage consumption with the feeding of more grain as cows of lower potential; the difference in total nutrient intake would, therefore, be accentuated over the difference in grain alone. With these combined effects, an increase of 10 lb. daily in mature equivalent FCM potential would result in 13.5 lb. more FCM on changing from all-roughage to grain at a rate of 1 : 3. In other terms, cows with a capacity for producing 12,000 lb. FCM would be expected to respond with 4,062 lb. more FCM than cows of 9,000-lb. capacity on making this change.

When adjustment was made for the different increases in grain feeding, the increase in FCM response per 10-lb. increase in potential was reduced from 13.5 to 8.5 lb., still large enough to be important. Another method of presenting the relationship of milk response, independent of differences in grain intake of the groups, is the ratio of the extra milk produced to the grain fed, comparing Rations H with O (Table 3). The correlation between this ratio and the potential was still very high, 0.88 ($P < 0.01$). This correlation would probably have been higher except for the aberrant value for group R8G, which may have been influenced by the two short lactations and much greater variability of the cows in this group. Considering only the Holstein groups, in which the data seem more reliable, cows with an average daily potential of 36.5 lb. (8,900 lb. 245-day total) yielded only 0.58 lb. milk per pound extra grain fed; whereas, the two groups with 47.9 lb. daily potential (11,700 lb. 245-day total) produced an average of 1.35 lb. extra milk per pound extra grain fed on the 1 : 3 ration.

There are several studies reported in the literature which support the law of diminishing returns in milk production, the most comprehensive study being the cooperative work reported by Jensen *et al.* (7). If such a law were to exhibit itself in this study, the response in pound milk per pound grain should be less for the second increment of grain, from M to H, than for the first, from O to M. In four of the groups this was observed, but in the four other groups the second increment produced more response than the first. These groups were equally divided between the two phases, but if the mean FCM and grain figures for the C phase were used, the ratio for the first increment was 1.20 and for the second, 1.21. In the R phase the ratio for the first increment was 0.87 and for the second 0.79, as would be expected in diminishing returns. The fact that the lower-potential cows gave more indication of the diminishing returns than the higher-potential cows is concordant with the results of Jensen *et al.* (7).

Since roughage intake declined with increasing grain, total energy intake also should be considered. However, the conclusions were the same when CNE values were used instead of pounds grain.

Since response to grain feeding has been shown here to depend to a large extent on the production potential or quality of the cows, it is not possible to make a blanket recommendation as to what level is most profitable. The relative costs of grain and hay and the price received for the milk produced also are of great importance. Without going into detail on cost aspects, it seems reasonable to

consider that additional costs of handling extra grain and extra milk might at least be covered by the decrease in roughage used. Thus, if the price received for the extra milk is at least as much as the cost of the extra grain fed, the grain feeding would be profitable. For the high-potential cows in this study, the cost of the grain could be 1.35 times as much as the price received for milk and it would still be profitable to feed the grain, assuming that under good management there would be no increase in health problems. However, for the low-potential cows (group R6H), the grain could only be 0.58 as expensive as the price of milk, to break even. With 4% milk at $5.50 per hundredweight, grain would need to be available at $3.19 per hundredweight, or $63.80 per ton, to be profitable for this group.

A third factor of great importance is the quality of the roughage, and perhaps this factor has not received adequate consideration in recommendations on levels of grain feeding. In the previous experiment, in which excellent quality hay (U.S. No. 1 leafy, green alfalfa hay) and corn silage were used (9), it was concluded that medium grain feeding would be most profitable under most conditions. In that study the average potential producing capacity of the cows, adjusted to a 245-day basis, was 8,500 lb., compared with 9,880 lb. potential of the cows in this study, but the quality of the roughage also was such that a higher level of intake was attained.

The all-roughage cows in the hay experiment consumed 2.29 lb. hay and 2.79 lb. silage, or 3.08 lb. hay equivalent per hundredweight, compared with a figure of 2.40 lb. hay equivalent per hundredweight in this study. If the law of diminishing returns is accepted, particularly for cows of lower potentials at the higher relative levels of feeding, then the response from grain feeding would be expected to be less when a larger amount of the nutrients is supplied by excellent quality roughage. This is borne out by the average responses for the two increments in the hay study of 0.71 lb. FCM per pound grain for the first and 0.32 lb. FCM per pound grain for the second. And this would, of course, lead to the conclusions drawn by the authors, that the medium level of grain feeding would be more profitable than the high-grain feeding.

In this study, comparisons of experimental production with potential production have not been calculated or discussed, because it seems likely that, from experiment to experiment, potential is determined at different levels of feeding and these values are not, therefore, really a satisfactory base for comparison. Even within an experiment, it is likely that cows of low potential have more nearly reached their maximum than cows of higher potential. If a cow is not producing as much as desired, it is often fed a little extra grain to try to bring it up; in contrast, in many cases some upper limit is placed on grain feeding in order not to force the high-producing cows with the result that their maximum is not likely to be attained. Even the use of a simple ratio of grain feeding inherently tends to overfeed the low producers and underfeed the high producers. Thus, the relationship demonstrated between response and potential was close, but the predictions not entirely unbiased.

Lactation curves. Since the rate of decline in production has been shown to

be proportional to the level of production, the curves of lactation have been presented on an arith-log basis to eliminate the influence of the different levels of production on the slopes of the curves. Separate curves were plotted for each lactation and for each phase and ration. The differences among the curves seemed to be attributable largely to the time during the lactations when the different cows went on pasture. Therefore, all lactations and both phases were combined for each ration (Figure 1); in this way, the differences in stage when going on

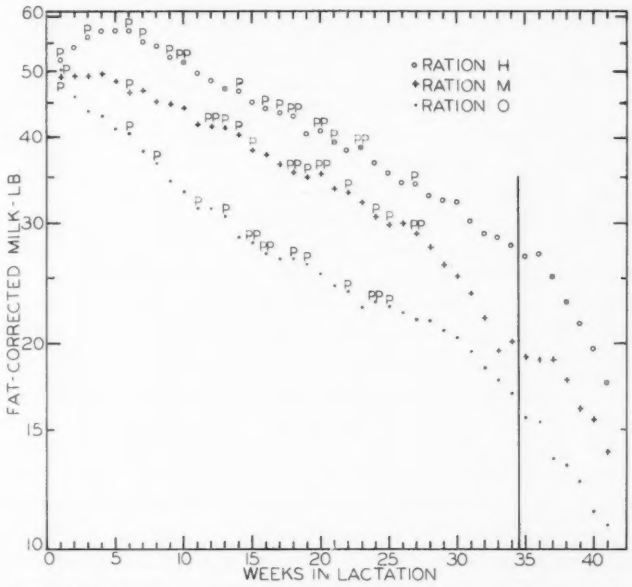

FIG. 1. Weekly FCM production by cows receiving no grain (Ration O, 21 lactations), medium grain (Ration M, 20 lactations), and high grain (Ration H, 21 lactations). The symbol P indicates that one cow went on pasture during that week.

pasture were largely balanced. However, the symbol P in the figures indicates that on Ration O, the curve was less steep when several cows were going on pasture and more steep at the lower end of the curve when no cows were going on pasture. This part of the curve, from 28 to 35 wk., should not have been influenced to this extent merely due to effects of gestation. The same observations apply to the Ration M curve, but very little to the Ration H curve.

The peaks of the curve were of greatest interest; the Ration O curve dropped sharply right from the beginning; the Ration M curve showed negligible increases to the fourth week and then declined; and the Ration H curve showed the accepted peak at 4 to 6 wk., followed by a steady decline. Thus, it was apparent that the extra nutrients from the grain on Ration H were necessary to secure greater production from the cows, but it can not be said that the maximum was attained.

Influence of pasture on lactation curves. The percentage decline, calculated on an instantaneous basis, comparing the 30 days before going on pasture with the 30 days immediately after, showed that the Ration O cows increased 6.9%, the Ration M cows declined 5.2%, and the Ration H cows declined 6.4%. The differences were significant ($P < 0.01$). The average monthly decline for 81 cows in this herd between the second and eighth months from another study was 11.5%. Thus, even those cows on high grain did respond favorably to going on pasture, since they declined only 6.4% compared with the average 11.5%.

It is commonly accepted that the response to pasture is greater in late lactation than in early lactation, and these figures support that view, the average regression of percentage response on weeks fresh being 0.80% per week ($P<0.01$). The within-ration-within-year regression of percentage responses to pasture on weeks fresh for Rations O, M, and H were 1.33, 0.82, and 0.42, respectively, but the differences were not significant.

Persistency. An index of persistency (12) was calculated for each lactation from Week 12 to 35, in which the persistency was 100 minus the percentage decline per 4-wk. period on an instantaneous basis, based on the short-cut orthogonal polynomial coefficient method (1). By eliminating the first 11 wk., the effects of adjustments to the ration, particularly in regard to body condition, were reduced. Including estimates for missing values, the persistency values for rations O, M, and H in Phase C were 95.2, 90.4, and 90.8%, whereas in Phase R they were 85.3, 84.7, and 88.7%. This inconsistency can be taken as an indication that after the first 11 wk. the ration had little influence on the persistency of production. The important differences in the lactation curves took place in the first few weeks of lactation.

Body weight. The changes in body weight are presented graphically (Figure 2) by months during the experimental lactations. In addition, the last weights before calving are presented, as well as values interpolated to be 60 days before calving, for comparison purposes. Only completed lactations were included. The smooth curves were plotted as the cubic curves fitted to the data. Because of the close fit, extrapolation to day of calving seemed permissible and showed the similarity of the groups with regard to body size, as also shown by the precalving weights.

Since only completed lactations were used, there was some imbalance with regard to the level of feeding in the lactation previous to the lactation as plotted. However, this had relatively little effect on the changes. Comparing the last weight before calving with the first weight after calving, the O cows lost 218 lb., the M cows 162 lb., and the H cows 198 lb. Schmidt and Schultz (21) also showed relatively little relationship between the level of feeding previous to calving and the loss in weight on calving.

The changes on Rations O, M, and H from the first to fourth month were −106, −19, and +16 lb.; from the fourth to the tenth month they were +190, +109, and +142 lb. Thus, the decline in the O lactations was marked in the early part, but the rate of gain later was even greater than in the M or H lactations. Some of the O cows became extremely thin, as would be expected of potentially

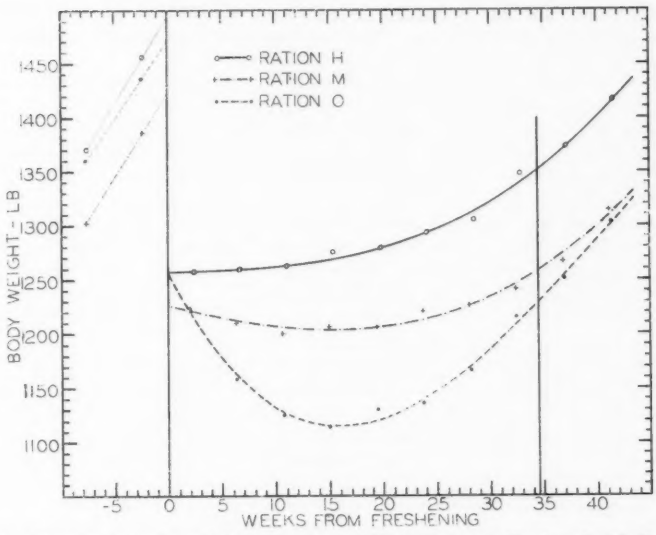

FIG. 2. Changes in body weight of cows receiving no grain (Ration O, 15 Holstein, six Guernsey), medium grain (Ration M, 14 Holstein, six Guernsey), and high grain (Ration H, 15 Holstein, six Guernsey). Values previous to calving were averaged according to the ration received during the following experimental lactation; the same cows are represented by a symbol before and after freshening.

high producers consuming inadequate amounts of nutrients. If some of the loss in body weight was due to protein loss, it is conceivable that the regained protein tissue would require less energy than fatty tissue. It has also been suggested that animals on low intakes may become more efficient in nutrient utilization; if true, this would permit greater body gain from the same nutrient intake. However, the failure of the M group to follow the pattern makes such conjectures less plausible, with the possibility that the differences were due only to sampling error.

Comparison of weights 60 days before freshening with those in the tenth month showed the changes in the O, M, and H lactations to be −57, +12, and +46 lb., respectively; in the O lactations, cows failed to regain their precalving weights in a total period of 50 wk.; whereas, the H cows had more than regained previous body weights. Whether some of the gain of the H cows was growth or entirely condition is not clear but since, on the average, the cows were in their fourth lactations, it seems unlikely that much of the change in weight was growth tissue.

Energy relationships for this study will be presented in a later paper.

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EFFECT OF INITIAL LEVEL OF MILK PRODUCTION ON RESPONSE TO FORAGES OF VARYING QUALITY

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SUMMARY

The persistency of milk production was found not to be related to the initial level of milk production in a 42-day trial, using eight cows with different levels of production, allowed only good pasture. On a within-period basis, the respective correlations between level of actual and 4% FCM and persistency of milk production were −.007 and −.097. On a within-cow basis, the respective repeatabilities for actual and 4% FCM were .373 and .367. When the data were corrected for period effects, the repeatabilities were .810 and .654.

Using data from several feeding trials with pasture and silage, the relative persistency of cows at varying levels of milk production was studied when cows were fed forages of different qualities. Within forage quality, the correlations between initial milk production and percentage change in milk production ranged from −.067 to .251. None approached significance. When the data were studied by regression techniques, there was evidence that low-quality forages were more detrimental to high producers than to low producers. High-quality forages were equally capable of maintaining milk production on cows producing 20 to 50 lb. of milk per day.

The amount of concentrate which should be fed to milking cows to supplement free-choice forage rations has been of interest to dairymen and research workers for many years. Most suggested feeding programs have assumed that dairy cows can consume only so much forage and, thus, that their concentrate requirements are in direct proportion to milk production. A frequently quoted rationing system for European dairymen is to feed forage for maintenance and the first gallon of milk and concentrates for the remainder of the milk produced. The common American system of feeding forage free choice and concentrates at 1 lb. of grain per 3 or 4 lb. of milk is based on a similar assumption, although it places greater emphasis on forage at wide ratios. The American system also includes an increase in TDN from concentrates for high producers. Thus, a 1,000-lb. cow being fed grain 1 : 4 on pasture would receive 20% of its calculated TDN requirement from grain when producing 20 lb. of milk and 30% of its TDN from grain when its production was 40 lb. per day.

As a result of improved techniques in pasture evaluation, which have permitted the calculation of daily dry matter intake for free-grazing cows, several workers have shown that dry matter intake increases with increasing level of milk production. This work was recently reviewed by McCullough (3) and need not be reviewed here. However, if these findings are correct, then cows at varying levels of milk production should give proportionate changes to similar forage conditions. Thus, cows varying in milk production from 20 to 40 lb. per day should give a similar percentage change in milk production when grazed on

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the same pasture. The data in this paper represent one approach to the testing of this hypothesis.

MATERIALS AND METHODS

For the convenience of the reader, the data have been divided into two parts, one called uniformity trial and the remainder listed as feeding trials.

The data for the uniformity trial were collected over three consecutive 2-wk. periods. Eight Guernsey cows varying in daily milk production from 13 to 36 lb. (all between 2 and 6 mo. in lactation) were grazed on a mixture of oats and ryegrass without supplemental feed. The forage was high in quality, as indicated by dry matter digestibility, but it was not possible to maintain a surplus of forage throughout. Period comparisons in milk production were made as an average of the last seven days of milk production in each period and dry matter digestibilities from grab samples taken on one day in the last week of each period. Dry matter digestibility was determined by the chromogen technique as outlined by Reid *et al.* (4).

The data for the feeding trials were taken from trials conducted at this Station, in which grain was fed on the ratio basis. The cows used were Jersey, Guernsey, and Holstein from the Station herd. The pasture trials included a 14-day experimental period, and production response was calculated as a percentage response of each cow in terms of a seven-day pre-experimental period. All data are in terms of 4% FCM. Since the data were collected over several grazing seasons, the pre-experimental feeding was not constant. The program followed was to use pasture similar to the experimental lots for the pre-experimental period. If the pre-experimental period is conducted on the same lot which is used for the experimental periods, the forage being studied is obviously not fresh pasture but one that has been grazed before the trial. Although there is no perfect solution to this problem, this procedure appears valid. Grain was fed at a ratio of 1 : 4. The silage trials were all 28-day experimental periods with silage fed free choice and grain fed on the grain-milk ratio basis. Ratios of 1 : 3, 1 : 4, and 1 : 6 were fed, depending upon the silage. Included in the trials were several pasture types and commonly used silages.

In both the uniformity and feeding trial data, the average 4% FCM produced during the last seven days of the experiment was calculated as a percentage of the average production during the seven-day pre-experimental period. This percentage was termed persistency of milk production and afforded a basis for comparing cows at various levels of milk production. The length of the experimental periods is considered adequate to measure differences in feeding value but not changes due to such long-time effects as minor nutrient deficiencies, lowered palatability due to long feeding trials, or similar problems. Blaxter (1) found that 60–70% of the ultimate response to ration changes was reached in 1 wk. and 90% in 2 wk. When 3 wk. had elapsed, no additional response could be detected. The use of such data seems valid if the data are thought of as field trials in which there was an indirect comparison of feeding standards for many forage conditions.

RESULTS AND DISCUSSION

Uniformity trial. The data from the cows by periods are shown in Table 1. The total drop in milk production was greater than would be expected from advance in lactation, which probably reflects the below-optimum quantity of forage. The high quality of the forage is reflected in the dry matter digestibilities, which average above 70%. In Table 2 are shown the relationships between the cows. On a within-period basis, the respective correlations between level of actual and 4% fat-corrected milk (FCM) and persistency of milk production were −.007 and −.097. This lack of correlation indicated that on a percentage basis the high-producing cows were just as able to meet their nutrient needs as were the low producers. Although not shown in the tables, body weight changes were similar for all cows over the 6-wk. period. The repeatability data provide some measure of the individuality of the cows. On a within-cow basis, the respective repeatabilities for persistency of actual and 4% FCM were .373 and .367. When the data were corrected for period differences, the respective repeatabilities were .810 and .654. Thus, the individual cow was rather constant in its response to the environmental changes reflected in the period differences. This response to a single ingredient ration is somewhat different from the significant cow × period interactions observed by Burt *et al.* (2), when their cows were offered rations comprised of three feeds. It is of interest, although not unexpected, that there was no within-cow repeatability for dry matter digestibility. This lack of repeatability probably reflects the tendency of cows to alter their grazing habits and forage selectivity from period to period and day to day. The respective coefficients of variability for milk production are typical for this factor in dairy cows. The relatively small coefficients for individual cows are further indication of the general response at each level of milk production. The respective between-cows, within-period coefficients of variation for actual and 4% FCM were 11.3 and 11.8%. The large coefficients exhibited by the animals as a group are some indication of the variation between cows, which can not be included in general feeding recommendations but must be corrected for by the dairyman for his own herd.

TABLE 1

Actual and fat-corrected milk production and dry matter digestibility of cows on uniformity trial

Cow No.	Period 1			Period 2			Period 3		
	Milk/day		D.M. dig.	Milk/day		D.M. dig.	Milk/day		D.M. dig.
	Actual	FCM		Actual	FCM		Actual	FCM	
	(lb.)		*(%)*	*(lb.)*		*(%)*	*(lb.)*	*(%)*	*(%)*
1	36.3	41.7	76	33.3	37.3	74	29.2	36.2	78
2	32.6	33.6	77	29.9	34.4	76	29.4	33.4	75
3	35.5	38.7	77	27.7	29.4	77	26.9	27.3	78
4	18.1	18.6	75	16.8	20.8	78	13.7	16.9	79
5	27.3	28.5	73	24.1	24.1	78	21.8	22.0	79
6	21.9	24.5	71	20.9	23.7	77	18.4	21.0	77
7	13.0	14.4	73	10.8	13.1	75	10.5	12.0	75
8	26.5	30.5	69	24.2	28.6	75	23.9	28.4	79

TABLE 2
Repeatability of milk production and dry matter digestibility of cows on uniformity trial

Item	Repeatability	Coefficient of variation: Over all cows and periods	Coefficient of variation: Within cow and periods
		(%)	
1. Persistency of actual milk production	.373*	13.7	11.0
2. Persistency of 4% milk production	.367*	12.7	10.3
3. Persistency of actual milk (corrected for periods)	.810**	10.9	4.9
4. Persistency of 4% milk (corrected for periods)	.654**	10.9	6.6
5. Dry matter digestibility	0.0	3.3	3.5
6. Dry matter digestibility (corrected for period)	.03	2.7	2.6

Correlation of actual milk production and persistency of actual milk within periods = − .007
Correlation of 4% FCM production and persistency of 4% FCM within periods = − .097

* Significant at the 5% level.
** Significant at the 1% level.

Feeding trials. The data used were from feeding trials in which cows of varying levels of production had been used to evaluate the feeding value of either pasture or silage. There were 69 such cow observations on pasture and 66 observations on silage. The pasture data were grouped according to observations on which all cows had exhibited an average persistency of milk production of less than 95%, 95 to 105%, or above 105%. Similar groupings for silage were below 85%, 85 to 100%, and above 100%. These average persistencies in milk production for all cows on the treatment were referred to as lot persistencies. Since this average change in milk production reflects all changes in the ration and their effect on production, it is a commonly used measure of forage quality and is so referred to in this paper. In Table 3 are shown the correlations *(r)* between initial levels of milk production and individual cow persistencies within the six groups. The corresponding regression coefficients *(b)* also are listed. None of the correlations or regression coefficients approached significance. This indicates that initial level of milk production was not a significant factor in determining the percentage response in milk production of cows on similar qualities

TABLE 3
Relationships between initial milk production and persistency of production within forage qualities

Lot persistency	No. cows	Initial production × cow persistency	
Pasture *(%)*		*(b)*	*(r)*
Below 95	29	−.116	−.131
95–105	12	.175	.151
Above 105	28	−.108	−.067
Silage *(%)*			
Below 85	20	−.573	.251
85–100	25	.070	.030
Above 100	21	−.997	−.293

of pasture or silage. The data were regrouped into four levels of initial milk production and correlations determined between the persistency of milk production of individual cows and the lot average of all cows on the forage. These data are shown in Table 4. There was no significant relationship between initial level of milk production and response within forage qualities (Table 3). Thus, cows producing 40 lb. of milk per day had an 88% persistency of milk production when grazed on a pasture with a lot persistency of 90%, whereas cows with an initial production of 20 lb. of milk had an average persistency of 89%. It should be noted, however, that in pounds of milk the higher-producing cows would exhibit a larger change per unit of time. On high-quality forage which maintained or improved average milk production, the higher-producing cows responded as well as low-producing ones. On silage there was a tendency for the higher-producing cows to exhibit a lower persistency of milk production on all qualities of silage. The latter response may be due to the frequently observed difficulty in securing high dry matter intake of silage as compared with pasture and hay.

TABLE 4

Relationships between initial production and persistency of milk production within levels of milk production

Initial level of production	No. of cows	b[a]	Cow persistency when lot persistency was					r[a]
			70%	80%	90%	100%	110%	
Pasture *(lb.)*								
Above 40	7	1.260	....	....	88	101	113	.83*
30–40	11	1.043	....	....	92	103	113	.82**
25–30	17	0.853	....	....	89	98	106	.76**
20–25	16	0.680	....	....	94	100	107	.50**
Under 20	18	1.135	....	....	89	100	•112	.74**
Silage *(lb.)*								
30–35	16	.855	70	79	87	96	106	.73**
25–30	17	.878	71	80	88	97	106	.76**
20–25	24	1.045	71	82	92	102	113	.73**
Under 20	8	.831	76	84	93	101	109	.67

[a] Where Y = Persistency of cow.
X = Persistency of all cows on forage.
* Significant at the 5% level.
** Significant at the 1% level.

In Table 5, the data from Table 4 were reconverted from milk persistency percentages to a 4% FCM milk production basis. Since none of the pastures was poor enough to give only 70% persistency of milk production, direct comparisons were possible only at the 80, 90, and 100% levels of lot persistency. When all cows had an average persistency of milk production of 80%, the cows with an initial production of 35 lb. produced 29 lb. of milk per day, whereas those with an initial production of 20 lb. produced 16 lb. on pasture. The same animals had an expected production of 36 and 20 lb. when the lot average was 100%. Cows on silage produced slightly less at all levels of forage quality.

On both pasture and silage, some forage conditions resulted in an increase in milk production in cows at all levels of initial production. This ability to improve production in low-producing cows is in agreement with the observation

TABLE 5
Changes in fat-corrected milk production within levels of initial production

Initial milk production	Type of feed	Milk production maintained when persistency of all animals averaged			
		70%	80%	90%	100%
(4% FCM)		*(lb. per day of 4% FCM)*			
40	Pasture	....	30	35	40
40	Silage	....	....	....	....
35	Pasture	....	29	32	36
35	Silage	25	28	30	34
30	Pasture	....	24	27	29
30	Silage	21	24	26	29
25	Pasture	....	22	23	25
25	Silage	18	21	23	25
20	Pasture	....	16	18	20
20	Silage	16	17	19	19

of Burt *et al.* (2), that there was no tendency for response in milk production to diminish with advance in lactation when account had been taken of initial milk yield. This statement may appear at odds with the well-known effect of advancing lactation on milk production. Turner (5) found that the net capacity of the udder declined during lactation in proportion to the concurrent decline in daily yield. The decline in maximum yield was contributed to, jointly, by decline in physiological capacity and decline in secretion pressure. In early lactation, decline in secretion pressure contributed most to decline in physiological capacity, with loss in physical capacity becoming important only in late lactation. Thus, in these experiments, the cows apparently had lost only their lactation pressure and not secretory tissue, with the result that improved nutrition was able to improve production in proportion to their level of milk production when placed on the forage.

Two general conclusions appear valid from the data. First, a silage fed free choice, that will not sustain milk production on a percentage basis when fed to low producers, will be even more detrimental to higher producers. Secondly, a forage capable of sustaining normal milk production in cows producing 20 lb. of milk per day can reasonably be expected to do equally well with cows producing 40–50 lb. per day when grain is rationed on a grain-to-milk ratio basis.

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EFFECT OF RAPID GROWTH WITH FATTENING OF DAIRY HEIFERS ON THEIR LACTATIONAL ABILITY

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SUMMARY

Seven pairs of identical-twin heifers were separated at ages varying from 4 to 12 mo. One of each pair was fed a normal control ration and its mate was fed heavily on concentrates until first calving to produce rapid growth and fattening. At 2 yr. of age, the fattened heifers weighed 32% more than the controls. After their first calving they were fed alike. Only one of the seven fattened twins produced more than its mate in the first lactation. The average FCM production by the fattened heifers in the first lactation was 84.8% of the controls. The average production difference between the rapidly grown and normal twins remained in the second lactation, but individual pairs reacted differently in the two lactations. Udder structure of three of four of the fattened twins examined was abnormal, with areas lacking development of secretory tissue. Weight changes during the first lactation indicated differences in metabolism between the fattened and control twins which may have affected lactation.

Dairy heifers are usually reared and bred so that they calve for the first time between 2 and 3 yr. of age. Occasionally, the theory is presented that by accelerating growth through heavy feeding the age of first calving might be advanced, and that the earlier production from such heifers would offset the extra cost of rearing. This theory has been tested in an experiment with Holstein heifers by Herman and Ragsdale (3, 4). They found that rapidly grown heifers produced less milk than expected in the first, second, and third lactations. Recent information from other growth experiments has been presented in a review by Burt (1), in which there is general agreement that no benefit to lactation results from heavy feeding of heifers. Experiments with identical twins have given the most reliable evaluation of effects of growth upon lactation, because they have not been complicated by unknown genetic differences in lactation potential. Reports from Hanson (2), and a preliminary report by Swanson (7) of work with twins, have confirmed the early Missouri reports that overfeeding heifers inhibited lactation. Reports from a similar study at Cornell, using nontwin Holsteins (6), have shown neither a reduction nor an increase of lactation in the heavy-fed heifers.

This study was made to obtain further evidence of the effects of rapid growth with fattening upon lactation, especially when measured with identical-twin heifers having a predominance of Jersey breeding.

EXPERIMENTAL PROCEDURE

The complete experiment was conducted over a period of 6 yr. One group of twin heifers was started in 1951 and another in 1954. The twins were secured from the area around Knoxville, Tennessee. They were all from dairy cows and sired by dairy bulls. Two pairs were registered Jerseys, five pairs were 50%

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or more Jersey blood, and one pair was 25% Jersey and 75% Holstein. As nearly as could be determined from phenotypic characters, they were all identical twins. Blood types for cellular antigens were identical within all pairs and no evidence of blood admixture within twins appeared.

Because the heifers differed in age when secured, they were started on the experiment at different ages. Five pairs were started at 3 to 4 mo., one at 7 mo., one at 11 mo., and one at 12 mo. Those started at 7 to 12 mo. were markedly undersized for their age when purchased.

One of each pair was put in a control group and one in a heavy-fed group. The latter heifers were given nearly all of the concentrates they would readily consume, to promote the desired rapid growth and fattening. Good-quality mixed hay and a mixture of salt and bonemeal were also available. The control group was fed a limited amount of grain up to 10 to 12 mo. of age. After that, they were generally fed only roughage plus salt and bonemeal. The roughage was hay, silage, and pasture, according to seasons and supplies, and was quite variable in quality. No attempt was made to feed all heifers the same quality of roughage.

The heifers which started the experiment at 3 to 4 mo. were bred to calve at 24 mo. The ones started at older ages were bred later, and calved at 26 to 29 mo.

The first group of heifers was group-fed, and complete feed consumption data were not kept. The last four pairs of twins were individually fed for most of the experimental period and consumption of grain and hay was determined.

Body weights were secured at weekly intervals. Heart girth and height at withers were also determined weekly through the first lactation, and after that time at monthly intervals.

After calving, a schedule of concentrate allowance was set for each pair of twins so that at comparable stages of their lactations they were fed the same amount. Roughage consumption was unrestricted; and, except for brief periods when all feed was given in the barn, it was not determined. The feed allowances during lactation were purposely set above the common feeding standards so that no restriction of milking potential could have been caused by poor feeding in the lactation.

The twins were fed, handled, and milked in pairs after calving. They were milked by machine twice daily, and all milk weights were recorded. A composite daily sample was taken once each week and analyzed for butterfat and total solids.

The twins of each pair were bred to calve as closely together as possible so they could be compared in their second lactations. Not enough pairs remained sufficiently alike for adequate comparisons in third or later lactations. At the end of their second lactations, four of the pairs were slaughtered and their udders were removed. These were fixed by intramammary injection of formalin, frozen, and sawed into sections for gross inspection of udder structure.

RESULTS

Feed consumption. Appetite for concentrates varied widely. One in the first group of fattened heifers apparently ate almost no hay and, as a result,

developed blindness due to vitamin A deficiency, even though it was the fattest heifer in the group. After the animal became blind, it had difficulty getting around; therefore, it and its mate were discarded from the experiment. The four individually fed, fattened heifers ate an average of 9.6 lb. concentrates and 5.3 lb. hay per day for a 90-wk. period—up to first calving. From 28 to 87 wk. of age inclusive, the estimated TDN consumption by the fattened heifers was 153.5% of that by the controls. The average daily feed consumption by the controls from 49 to 67 wk. was 1.7 lb. grain and 9.9 lb. hay. From 68 to 86 wk. they consumed 12.7 lb. alfalfa hay daily on ad lib. feeding. After 87 wk. they were pastured or group-fed. This consumption provided less than the recommended allowance for growing heifers. Their appearance was more lean than usually considered normal; so the controls in this experiment may be considered slightly underfed.

Growth. The twins were nearly alike in weight, within pairs, when they were started on experiment. Average increases in body weight before first calving are shown in Figure 1 for the seven pairs used in lactation comparisons. The standard for normal Jerseys (5) is also shown. The controls were below the standard at all ages shown (11–24 mo.). The fattened heifers were above the controls and the standard at 11 mo. They remained above, but parallel, to the standard until about 14 mo. After this age they gained rapidly. At 24 mo. the fattened heifers averaged 899 lb., which was 123% of the standard, and the controls averaged 683 lb. The fattened heifers weighed 132% of the controls at 24 mo. Heart girth data followed weights very closely, and are not presented here. Average increases in height of the seven pairs used in lactation comparisons are shown in Figure 2 for 11 to 34 mo. The fattened twins averaged 0.5 in. higher than the controls at 11 mo., 1.2 in. higher at 24 mo., and 1.0 in. higher

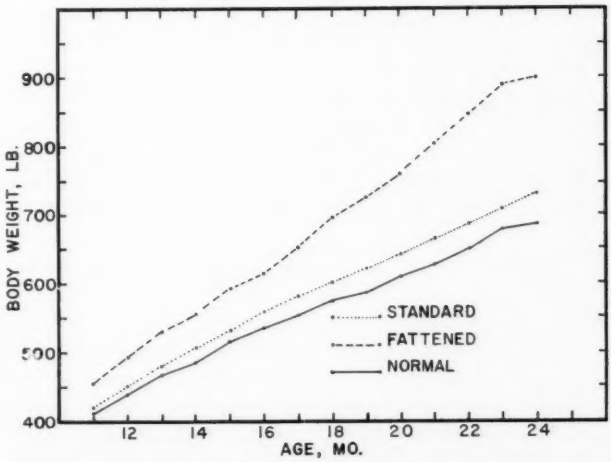

FIG. 1. Average growth curves of seven pairs of identical twins, comparing fattened and normal dairy heifers, and Missouri Standard.

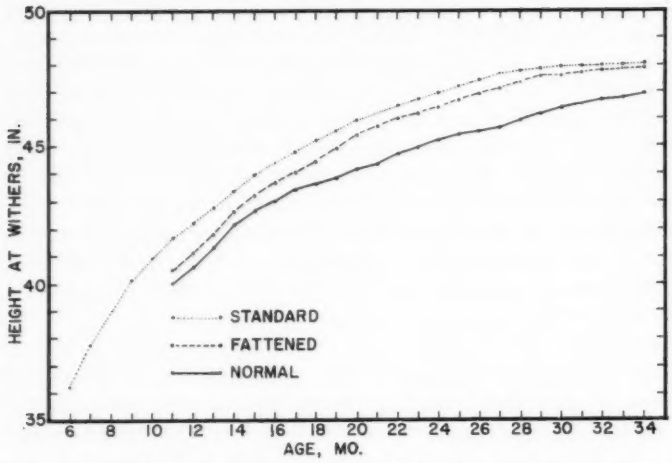

FIG. 2. Average height at withers of seven pairs of identical-twin dairy heifers, comparing fattened and normal rearing, and the Missouri Standard.

at 34 mo. The fattened twins did not average as high as the standard at any age. This indicates that the heifers used in this experiment were inherently smaller in frame (size) than the standard.

Average weight changes before and after calving are shown in Figure 3. Since the heifers calved at different ages, these are not the same as the age-weight changes (Figure 1). Before calving, the fattened twins averaged 964 lb.

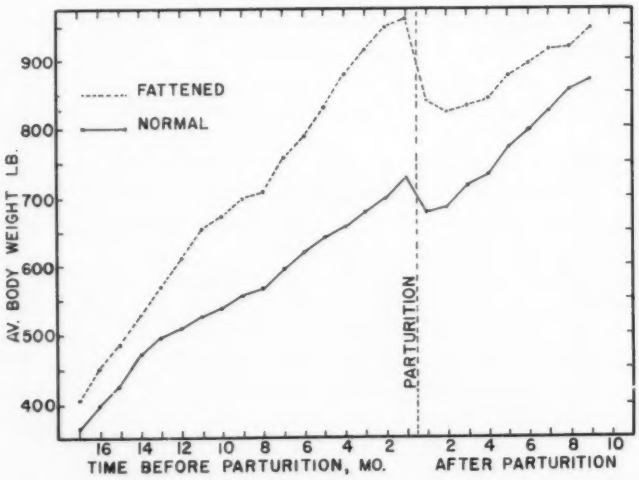

FIG. 3. Average body weight changes of seven pairs of fattened vs. normal-lean identical twins from 17 mo. before to 9 mo. after parturition.

and the controls 727 lb. By the next monthly weighing (about 2 wk. post partum) the fattened twins had lost an average of 143 lb., compared to a loss of only 50 lb. by the controls. At 9 mo. post partum the fattened heifers still lacked 18 lb. of attaining their prepartum weight, but the controls had gained 142 lb. more.

A summary of the weight and height changes of the individual heifers is presented in Table 1.

Lactation. The ages of calving are presented in Table 2. All of the pairs but one calved at comparable ages for the first lactation. The lean heifer of Pair 7 calved May 30 and the fattened one calved November 18. These differences in age and season could not be adequately corrected. After 10 wk. of lactation, the fattened heifer, which started at a lower level of production than the control, was more persistent in production. For this reason only the first 10 wk. yields were used in the comparison of this pair. Pair 3 did not start their lactations in comparable health; and from the breeding history and the lactation curve, an abortion was suspected in the fattened twin. For this reason, the lactation comparison of this pair was stopped at 22 wk. All other pairs appeared to be in normal health and condition throughout their full first lactations.

Results of comparable first lactation periods are given in Table 3. Only one of the fattened twins produced more than her mate in these comparisons, and that pair was noticeably affected by health problems. Four of the control twins produced considerably more than their mates. The fat tests of the fattened twins averaged slightly higher than the controls, but their solids-not-fat tests averaged slightly lower. Butterfat yields and fat-corrected milk (FCM) followed milk yields closely. The FCM yield of the fattened heifers averaged 84.8% of the FCM yield of the controls. Part of this difference was due to the average 20-day shorter lactation (all due to one pair) of the fattened heifers.

The average total lactation curves to 35 wk. are presented in Figure 4. The data for Figure 4 include the full lactations of Pair 7 with no correction for age or season of freshening, which gives a slight advantage to the fattened group. The greatest difference was in the first 20 wk. of lactation. Heifers that milked longer than this tended to produce alike near the end of the lactation.

In the second lactation, Pairs 5 and 6 were not comparable, because of premature calvings and poor health. The other five pairs were apparently normal and calved close together. Pair 3 was nearly 4 yr. old at this comparison (Table 2). The fattened heifers in Pairs 3 and 4 had much longer dry periods than their mates.

Milk production in the total second lactations is shown in Table 4. The comparative results are very similar to the first lactation, with the exception of Pair 4. The fattened heifer of this pair nearly doubled her previous lactation, while the control heifer unaccountably failed to milk as well as she had in the first lactation. In Pairs 1, 2, and 7, the control heifers produced more milk and milked more persistently than their mates, just as they had in the first lactation.

The average second lactation curves for 35 wk. of the two groups are presented in Figure 5. The second lactation followed very closely the pattern of the first lactation. Data were secured in third lactations for Pairs 3 and 4, and they milked nearly alike within pairs.

TABLE 1

Body weight and height of withers of fattened and control identical-twin heifers at 24 mo. of age, and weight changes from 11 to 24 mo. and from calving to 9 mo. postpartum

Pair No.	Weight gain 11 to 24 mo.		Weight at 24 mo.		Height of withers, 24 mo.		Weight at first calving		Weight at 9 mo. lactation	
	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean
	(lb.)				*(in.)*		*(lb.)*			
1	470	353	1,011	831	46.8	47.0	1,011	831	1,052	980
2	395	322	835	720	43.5	43.5	840	730	938	908
3	365	255	900[a]	740[a]	45.5	45.5	890	731	970	928
4	609	300	1,054	730	49.8	48.0	1,152	816	1,050	965
5	525	243	800	518	45.5	43.0	932	600	810	750
6	396	220	834	622	47.2	45.0	846	693	865	780
7	360	194	858	620	46.5	44.3	1,075	690	940	788
Av.	446	270	899	683	46.4	45.2	964	727	946	871

[a] Extrapolated from precalving weight because heifers calved before 24 mo. old.

TABLE 2

Ages at which heifers were started on experiment and calved for first and second lactations

Pair No.	Age started on experiment	Time fed differently	Age at first calving		Age at second calving	
			Fat	Lean	Fat	Lean
	(mo.)					
1	4	20	24	24	37	38
2	4	21	25	25	36	36
3	4	19	23	23	46[a]	46[b]
4	11	15	26	29	42	40
5	12	16	28	28	40	40[c]
6	7	19	27	26	38	38
7	4	21	31	25	42	41

[a] Second normal calving delayed because of early abortion terminating second gestation.
[b] This heifer calved normally at 34 mo. for second calving, but for comparison with her mate's second lactation, the third normal calving is used.
[c] Heifer aborted, resulting in abnormal second lactation.

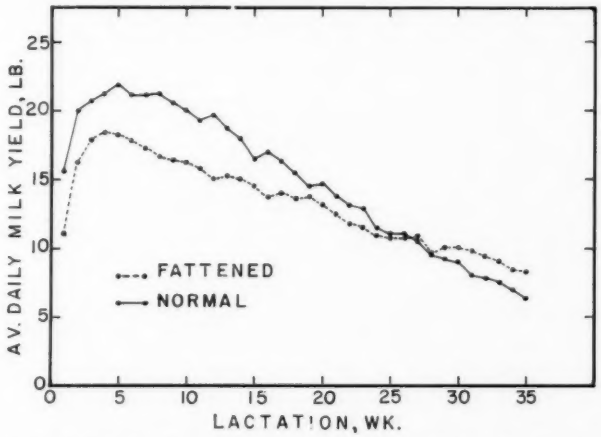

FIG. 4. Average lactation curves of seven pairs of identical twins, comparing fattening vs. normal feeding prior to first calving.

Udders. The poor production of the fattened heifers in the first two pairs led to a suspicion that udder function in these heifers was not normal. Near the end of the second lactation, while the heifers were still lactating normally, they were slaughtered and the udders examined as described above. The udders of the control twins were essentially normal in internal structure. However, the udders of the heifers which had been fattened contained areas of incompletely developed parenchyma on the periphery of the glands. The large open spaces were found to communicate with the milk ducts. This indicated that the ducts and framework of the gland were laid out, but that alveolar-secreting tissue had not filled the spaces provided. The most abnormal gland of this type is shown in Figure 6. Udders of Pairs 5 and 6 which did not have normal second lactations also were examined. The control heifers of these pairs had essentially normal udders. One of the heifers which had been fattened also had normal udder

TABLE 3

Comparison of first-lactation milk, fat, solids-not-fat, and 4% fat-corrected milk produced by fattened and control (lean) identical-twin heifers

	Days milked in comparison		Milk yield		Average fat test		Butterfat yield		Average SNF test		Milk yield 4% FCM		
	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	FCM diff.
	(No.)		*(lb.)*		*(%)*		*(lb.)*		*(%)*		*(lb.)*		
1	163	162	1,424	2,012	3.59	3.66	51	74	7.19	8.08	1,336	1,910	− 574
2	49	189	110	2,251	2.99	4.71	3	106	7.74	8.80	94	2,490	−2,396
3	154[a]	154[a]	2,894	2,087	5.16	5.28	149	110	9.00	8.61	3,397	2,488	+ 909
4	280	280	5,210	6,715	4.73	4.55	246	305	8.94	8.83	5,780	7,266	−1,486
5	308	308	4,384	4,502	5.44	5.33	239	240	9.15	9.36	5,334	5,399	− 65
6	280	280	5,549	6,107	4.63	4.56	257	279	9.03	8.93	6,071	6,621	− 550
7	70[a]	70[a]	1,297	1,389	6.40	6.27[b]	83	87	9.53	9.49	1,764	1,862	− 98
Av.	186	206	2,981	3,580	4.93	4.79	147	172	8.65	8.87	3,397	4,005	− 608

[a] Partial lactation, including only parts considered comparable.

[b] Fat tests were corrected for seasonal difference.

TABLE 4

Milk yields, fat tests, and 4% fat-corrected milk yields in comparable second lactations of identical twins reared differently before their first lactation

Pair	Days milked in comparison		Milk yield		Average fat test		Milk yield 4% FCM		FCM diff.
	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	
	(No.)		(lb.)		(%)		(lb.)		
1	100	140	1,367	2,567	3.69	3.67	1,303	2,438	−1,135
2	35	119	169	1,941	4.30	4.28	176	2,023	−1,847
3	259	259	5,062	4,894	5.10	5.01	5,896	5,634	+ 262
4	287	252	9,450	6,645	4.39	4.34	10,010	6,983	+3,027
7	203	217	2,204	3,853	5.89	5.56	2,829	4,751	−1,922
Av.	177	197	3,650	4,043	4.67	4.57	4,043	4,366	− 323

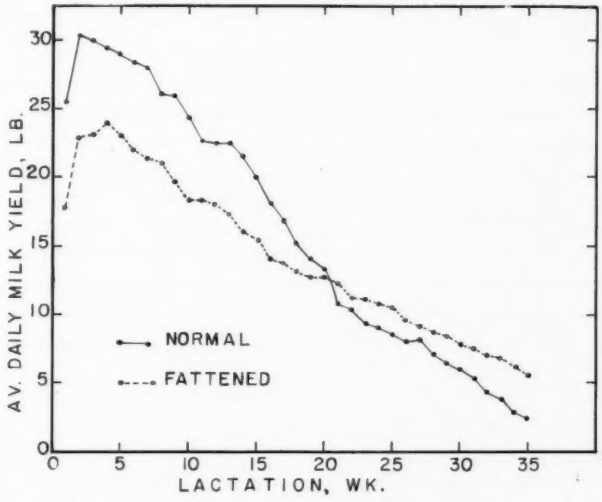

FIG. 5. Average lactation curves of five pairs of identical twins—second lactations of fattened vs. normal-growth heifers.

structure, but the other one showed the same deficiency as observed in the other fattened heifers. It may be noteworthy that twins of Pair 5, which were nearly alike in first-lactation milk production, were the ones not showing internal udder deficiency.

DISCUSSION

This experiment has demonstrated that in a large percentage of heifers heavy feeding to produce growth and fattening will result in poor lactation for one or two lactations. This confirms the earlier observation made by Herman and Ragsdale (3) with nontwin Holsteins and the observations of Hansson (2) from twins reared at different levels. In the Missouri experiment, early calving of

FIG. 6. Section of udder of fattened twin (Pair 2) showing incomplete development of the lobule-alveolar system.

the rapid-growth group and genetic variation may have contributed somewhat to the differences between groups. However, in this study and in Hansson's (2), ages of calving and inheritance were alike. The failure of the Cornell study (6) to show harmful effects in the fattened heifers may be due to breed differences or because the high-plane heifers were not as fat as those in this study.

The very poor lactations of some of the fattened twins in this study, compared to their control mates, and the lesser differences within other pairs, indicates that the effects of fattening may be much more serious in some heifers than others. Because of this variability in responses, the average differences in production were statistically significant for only the first 10 wk. of lactation. The fattened heifers logically could have been expected to produce higher, because of their greater size and superior nutritive condition. Although the numbers were small, there was an indication from this experiment that the heifers fattened from earliest ages, such as Pairs 1, 2, and 7, were more seriously affected than those fattened from the age of about 1 yr., such as Pairs 4, 5, and 6. The second lactation results emphasize this more than the first lactation.

The cause of the lactation deficiency can not be ascertained from this experiment alone. The deficient udder development observed could not explain all of the difference in yields as the fattened heifers still seemed to have enough secreting tissue to have produced better than they did. The weight changes during the first lactation indicate that there was a marked difference in body metabolism between the two groups. The fattened heifers lost weight in the manner of heavy-milking, mature cows after parturition, but they did not convert this loss to milk. The control heifers, with unfilled capacity for growth, grew rapidly throughout the lactation. It is postulated that the hormone stimuli resulting from the growth promotion in the control heifers enabled them to lactate better than their mates, which were growing very slowly at this time. Growth and hormone differences should not have been still affecting lactation in the second lactation, however.

Similar lactation effects have been reported between fat and lean primiparous rats (8, 9). It is a common observation of beef cattle breeders that fattened beef heifers do not milk as well as those kept in a normal condition during gestation. These observations indicate that the inhibitory effect of fattening on lactation may develop in other mammalian species.

In respect to economy of producing dairy heifers, these results indicate that very rapid growth (and possibly early calving) may be undesirable, because the added cost of feed during growth will not be compensated by extra lactation and may, in fact, be the cause of poorer than normal lactation.

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COMPARISON OF 6- AND 10-DAY COLLECTION PERIODS FOR DIGESTION TRIALS WITH DAIRY HEIFERS[1]

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SUMMARY

Six digestion trials (three animals per trial) were conducted with dairy heifers in a study of oat hay rations. Coefficients of digestibility were determined on the basis of both six- and ten-day collection periods, and coefficients of correlation were calculated for the two sets of values. Correlations for all nutrients except the ether extract fraction were significant at the 1% level of probability; the correlation coefficient for the ether extract fraction was not significant at the 5% level. When differences between values obtained at six and ten days were analyzed by means of the "t" test, none were found to be significant at the 5% level of probability. The largest loss of efficiency resulting from the use of a six-day collection period was −2.81% for the ether extract fraction. It appears that in this study there was little advantage in extending the collection period to ten days.

Digestion trials are expensive and time-consuming and, as a result, several substitute methods have been suggested to replace the total-collection procedure. In 1915 (1), it was suggested by a special nutrition committee that for ruminants preliminary periods should be ten to 12 days and collection periods, 12 to 15 days. Schneider and Ellenberger (6) have indicated the need for periods of seven to 30 days for adjustment and seven to 14 days for collection. Mitchell and Hamilton (4) and King (3) have shown that 14-day collections are adequate when compared to 20 or more days. Hamilton *et al.* (2) have indicated that seven-day collection periods were not as accurate as ten-day periods. The most recent study comparing shorter collection periods to standard periods was conducted by Staples and Dinusson (8), who used ten-day preliminary periods and compared seven- to ten-day collection periods. Losses in efficiency for the digestibility of the nutrients studied were less than 1.1% in all cases except that for nitrogen-free extract, which showed an efficiency loss of 6.83%. A recent study on the length of preliminary feeding periods was made by Nicholson *et al.* (5), who concluded that longer adjustment periods are needed when the hay-to-grain ratio fluctuates widely. The optimum period found for such trials lies between 16 and 30 days. For rations in which the basic components and the hay-to-grain ratio are constant, but protein supplement varies, a preliminary feeding period of seven days was found adequate.

Until recently, the majority of total collection trials have been of ten- to 14-day duration. During the past 2 yr. several publications have been released

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in which five- to seven-day collection periods have been used. It appeared that the shorter collection periods needed further study, and this experiment was undertaken to obtain data for comparison of six- and ten-day collection periods with dairy heifers.

EXPERIMENTAL PROCEDURE

Six digestion trials with three heifers each were used to compare the digestibility of rations containing oat hays cut both in bloom stage and milk stage. Throughout three trials each, one group of dairy heifers was fed bloom-stage hay (Trials 1, 3, and 5) and the other group milk-stage hay (Trials 2, 4, and 6). The hays were supplemented with cottonseed meal in Trials 1 and 2, with cottonseed meal and 3 lb. of molasses per heifer daily in Trials 3 and 4, and with cottonseed meal and 1 lb. of alfalfa meal per heifer in Trials 5 and 6. Each heifer consumed approximately 1 lb. of digestible protein daily, partly supplied by 0.9 to 1.2 lb. of cottonseed meal. Similar energy intake by groups and by pairs was obtained by varying the hay consumption from 11.0 to 16.0 lb. daily. The heifers weighed between 840 and 980 lb.

The total collection method was used and the chemical analyses were made according to A.O.A.C. standards. Duplicate samples of feces were taken for six days for the comparison of the six- and ten-day collection periods. Each collection period was preceded by a ten-day preliminary period. All the heifers had been fed oat hay rations seven days prior to the preliminary period of the first trial. Refused feeds were sampled and analyzed.

RESULTS AND DISCUSSION

The coefficients of apparent digestibility and total digestible nutrients of the rations are presented in Table 1. A survey of the data shows that, in general, the coefficients of digestibility for the six and ten days were reasonably close. The only trend noted was that the ether extract coefficients were higher in the ten-day periods than the six-day for 11 of 18 individual samples.

Coefficients of correlation were calculated for each nutrient, using the individual heifer data and also the averages. The correlation data are presented in Table 2. The correlations, with the exception of that for ether extract, were high for the digestibility data.

The average coefficients of digestibility were subjected to further analysis by the use of the "t" test. To show a significant difference between the six- and ten-day data, "t" values higher than 2.110 at the 5% level were necessary. The "t" values by nutrients were as follows: dry matter, 0.493; crude protein, 0.821; ether extract, 1.524; crude fiber, 0.777; N-free extract, 0.365; and TDN, 1.113. The digestibility data in the six- and ten-day collection periods for the ether extract were not significantly correlated, whereas the "t" test failed to show any significance for the differences between the two periods.

The average digestion coefficients for all the trials are presented by nutrients in Table 3, along with the standard deviations. The ether extract fraction showed the greatest difference in digestibility (2.15 percentage points) between the

TABLE 1

Coefficients of apparent digestibility and total digestible nutrients of oat hay–supplemented rations by 6- and 10-day periods

Trial	Heifer and length of period	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	TDN
		(%)					
1	G697— 6 day	64.75	56.14	68.13	68.80	65.64	62.88
	10 day	63.51	53.01	71.53	70.21	63.69	62.80
	H928— 6 day	57.88	49.34	64.28	62.79	58.51	56.70
	10 day	59.07	55.46	71.31	65.25	57.43	58.00
	X451— 6 day	61.89	51.30	67.18	69.28	60.97	60.31
	10 day	61.47	49.27	72.84	66.59	61.51	59.87
	Av.— 6 day	61.51	52.26	66.53	66.96	61.71	59.96
	10 day	61.35	52.58	71.89	67.28	60.88	60.22
2	G700— 6 day	58.07	50.48	69.70	59.38	60.38	57.28
	10 day	59.79	52.43	67.09	59.18	62.79	58.70
	H934— 6 day	60.01	47.94	66.75	59.83	65.06	59.39
	10 day	61.48	53.74	66.51	60.99	64.83	60.34
	X445— 6 day	64.91	54.92	72.39	66.99	67.50	63.80
	10 day	62.50	53.90	67.21	62.47	65.57	61.36
	Av.— 6 day	61.00	51.11	69.61	62.07	64.31	60.16
	10 day	61.26	53.36	66.94	60.88	64.40	60.13
3	G697— 6 day	61.00	50.87	69.33	61.03	63.71	57.85
	10 day	60.02	47.25	61.23	61.90	63.15	57.14
	H928— 6 day	57.71	46.66	65.59	57.88	60.50	54.76
	10 day	59.76	46.90	61.67	63.10	62.05	56.95
	X451— 6 day	58.61	46.08	70.73	57.04	63.25	56.21
	10 day	58.61	45.60	63.87	60.55	62.27	56.25
	Av.— 6 day	59.11	47.87	68.58	58.65	62.49	56.27
	10 day	59.46	46.58	62.26	61.85	62.49	56.78
4	G700— 6 day	61.36	48.83	69.31	56.36	67.91	60.31
	10 day	61.36	50.80	74.91	55.11	67.56	60.40
	H934— 6 day	61.53	46.45	65.62	59.34	66.72	60.08
	10 day	62.57	48.28	73.87	58.72	67.96	61.35
	X445— 6 day	62.25	49.40	67.24	59.51	68.29	61.30
	10 day	62.43	49.27	76.13	59.68	67.57	61.57
	Av.— 6 day	61.71	48.23	67.39	58.40	67.64	60.56
	10 day	62.12	49.45	74.97	57.84	67.70	61.11
5	G697— 6 day	61.54	58.65	64.68	67.96	59.06	59.37
	10 day	60.94	57.18	76.10	67.94	58.76	59.86
	H928— 6 day	61.02	56.16	64.35	64.67	61.91	60.01
	10 day	61.26	55.76	74.71	67.78	59.84	60.09
	X451— 6 day	58.11	52.12	60.16	66.83	54.98	56.17
	10 day	58.03	52.78	63.46	64.54	57.43	56.81
	Av.— 6 day	60.22	55.64	63.06	66.49	58.65	58.92
	10 day	60.08	55.24	71.42	66.75	58.68	58.52
6	G700— 6 day	55.96	48.37	56.02	60.53	57.57	55.88
	10 day	56.21	49.60	57.53	59.90	57.76	55.99
	H934— 6 day	58.97	54.29	62.48	62.48	62.10	58.41
	10 day	60.33	52.21	64.43	64.41	60.57	59.67
	X445— 6 day	61.80	56.95	59.26	64.61	62.90	60.84
	10 day	60.51	58.61	57.59	64.79	59.88	59.55
	Av.— 6 day	58.91	53.19	59.25	62.41	59.59	58.38
	10 day	59.02	53.47	59.85	63.03	59.40	58.40

TABLE 2
Coefficients of correlation of digestion coefficients between 6- and 10-day collection periods

	Individual heifers[a]	Average of heifers by trials
Dry matter	0.883	0.979
Crude protein	0.760	0.922
Ether extract	0.400	0.392
Crude fiber	0.828	0.915
N-free extract	0.914	0.995
TDN	0.906	0.973

[a] Coefficient of correlation of 0.575 was needed to be significant at 1% level and 0.456 at the 5% level.

TABLE 3
Comparison of the means and standard deviations of digestion coefficients by 6- and 10-day collection periods

	Dry matter		Crude protein		Ether extract	
	6-day	10-day	6-day	10-day	6-day	10-day
Mean	60.41	60.55	51.38	51.78	65.74	67.89
Standard deviation	0.38	1.21	2.98	3.18	3.89	5.93
	Crude fiber		N-free extract		TDN	
	6-day	10-day	6-day	10-day	6-day	10-day
Mean	62.50	62.94	62.40	62.26	59.04	59.19
Standard deviation	3.68	3.60	3.27	3.38	1.58	1.58

six- and ten-day means. Using this observed difference, it was calculated that ten heifer comparisons would have been sufficient to establish significant difference, whereas 18 comparisons were actually made.

Staples and Dinusson (8), in comparing seven- and ten-day collection periods, found very little loss of efficiency, except in the case of the nitrogen-free extract fraction, which was +6.834%. The largest loss of efficiency found in this study was −2.81% for the ether extract fraction. The loss in efficiency of the six-day periods compared to the ten-day periods was calculated as follows: The coefficients of variation (7) were calculated for the mean digestion coefficients of each nutrient representing the period lengths. The coefficients of variation of the six-day samples were subtracted from that of the ten-day samples and expressed in percentages. The losses of efficiency between the two lengths of collection periods were as follows: dry matter, −0.01%; crude protein −0.34%; ether extract, −2.81%; crude fiber, +0.17%; N-free extract, −0.19%; and TDN +0.01%.

The greatest error usually encountered in digestion trials is the irregularity in the quantity of feces excreted. This would account for a large part of the differences observed between the two lengths of collections in this study. Feed consumption can become a very important factor, especially if the forage is low in palatability.

It would appear from the examination of the individual data and the statistical analyses that the six-day collection periods in this study with dairy heifers

were as effective as the ten-day collection periods in estimating digestibility coefficients.

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INHERITANCE OF TWINNING IN A HERD OF HOLSTEIN-FRIESIAN CATTLE[1,2]

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SUMMARY

In a large herd of Holstein-Friesian cattle it was found that the sire and dam contribute to twinning tendency among the daughters. The data covered a 30-yr. period and involved 266 twinning cows (14%) out of 1,905 that calved one or more times. There were 313 multiple births among 7,387 calvings (4.2%). After adjustments for age, daughters of twinning cows twinned significantly more frequently than daughters from nontwinning cows. Parents which were themselves twins apparently transmitted twinning tendency no more frequently than if they had been singly born from the same ancestors. Twinning rates among paternal sisters from nontwinning dams were used to divide 23 sires into a low and a high group. Twinning rate among daughters (cow-basis) of the low-level sires from twinning dams was $11.7 \pm 2.6\%$. Similarly for the high-level sires, the rate was $23.3 \pm 3.7\%$. The difference between the two groups was significant ($P < 0.05$). Cows conceived within 75 days after cystic ovaries twinned at 15.1% of the subsequent calvings, as compared with 4.9% if conceived after 75 days ($P < 0.05$). Cows having twins only subsequent to cystic ovaries transmitted the tendency to their daughters at the same rate as cows twinning when not cystic. The total evidence indicates that twinning under most herd conditions is comparatively unimportant as an undesirable characteristic.

Hereditary control of multiple births in swine, sheep, and goats has been amply demonstrated. However, in a species such as the cow, multiple births are comparatively rare; thus, proof of hereditary control requires more complete and accurate calving records than are generally available. Further, the appearance of the trait frequently depends on the number of calvings by a cow and may possibly be modified by other physiological (1, 4) and environmental conditions.

Summaries of cattle twinning rates based on number of calvings reveal marked variations between breeds (5, 6, 12), herds within breeds (6, 8, 9, 12), and families within herds (8, 9). The total evidence favors hereditary control of the trait. Previous studies of breeding records from Carnation Milk Farms, Carnation, Washington, have shown the twinning rate to be substantially higher (3) than the combined averages for other Holstein herds (6). Because of the volume of data available (1,905 cows), it appeared feasible to investigate the

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hereditary aspects of twinning. The most pertinent results of this study are presented herewith.

EXPERIMENTAL PROCEDURE

The data were taken from the records of Carnation Milk Farms, Carnation, Washington, and cover 30 yr. (1920-50). The breeding records were most complete after 1925. Clinical data were collected by resident veterinarians, and breeding histories were maintained by one herdsman from 1925-50. Statistical probabilities were estimated using adjusted Chi-squares and confidence limits (10).

Twinning rate has been generally expressed as a percentage of total calvings. A second method is to express the rate in terms of total cows. Both methods were used in the present study.

Erb and Morrison (3) have also shown previously that in this herd only 1.3% of the 1,602 heifers gave birth to twins at their first calving (3). At consecutive later calvings, two through ten and over ten, the rates were, respectively, 4.4, 5.4, 4.7, 5.8, 6.1, 6.2, 5.9, 6.4, 7.2, and 4.1% (3). These rates were used to adjust for differences in age (based on calving number) when twinning rate was expressed as a percentage of the calvings.

For expressing twinning rates as a percentage of the cows, adjustment factors were calculated by dividing the number of cows twinning by each consecutively numbered calving by the total number of cows calving this number of times. For example, 1,602 cows calved at least once and 21 of these, or 1.3%, twinned by this time. Thus, for consecutive calvings of one through ten and over ten, the expected rates were, respectively, 1.3, 7.8, 13.8, 17.2, 21.0, 23.0, 25.3, 26.7, 27.6, 28.5, and 28.8%.

The age adjustments resulted in values, hereafter called expected rates, which reflected for this herd the rates that should have been observed for the average age at calving or average number of calvings by the cows involved if no other effects were involved. Therefore, comparisons between rates actually observed and those expected could be made with minimal bias due to age of cow. Also, twinning rates adjusted for differences in age were used. For example, if 25% of the daughters of a bull twinned and the expected frequency was 15%, then these daughters had more calvings than the average of all cows in the study, because the herd average was 14% (3). Then the age-adjusted rate would be 25% − (15% − 14%), or 24%. If the expected frequency had been instead 13%, then the adjusted value would have been 25% + (14% − 13%), or 26%.

RESULTS AND DISCUSSION

Of 1,905 cows which calved one or more times during the study period, 266, or 14%, had twins one or more times (3). There were 313 multiple births out of 7,387 observed calvings (3).

Relationship between twinning and cystic tendency. Clapp (1) previously noted that 9 of 24 cystic cases (37.5%) conceived twins subsequent to being cystic. Preliminary studies of the present data (4) indicated a similar trend.

TABLE 1

Relationship between cystic tendency and twinning rate

Days to conception from last observation[a]	Ovarian cystic follicles		Only short estrous cycles		Total	
	Calvings	Twins	Calvings	Twins	Calvings	Twins
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
0–25	37	16.2	240	4.6	277	6.1
26–50	32	12.5	148	8.1	180	8.3
51–75	24	16.7	123	4.9	147	6.8
Subtotal	93	15.1	511	5.7	604	7.1
76–150	61	4.9	236	6.4	297	6.1
> 150	61	4.9	216	3.2	277	3.6
Subtotal	122	4.9	452	4.9	574	4.9
Study total	215	9.3[b]	963	5.3[c]	1,178	6.0

[a] Of cystic follicles or termination of short estrous cycles.
[b] Expected rate, 5.1%.
[c] Expected rate, 4.6%.

Table 1 shows that twining was significantly more frequent at calvings following cystic ovaries (9.3% vs. an expected rate of 5.1%) than following short estrous cycles of 15 days or less (5.3% vs. an expected rate of 4.6%). Short estrous cycles occurring in the same reproductive period with observed cystic follicles were summarized under the latter group. The slight increase in twinning following only short estrous cycles was not significant.

Cystic cows averaged 15.1% twin births at calvings when conceived within 75 days after cysts were last observed, as compared with 4.9% when conceived after 75 days (adjusted Chi-square = $P < 0.05$). Though it appears desirable to avoid twinning (3, 9), it is questionable whether the disadvantages of twinning are sufficiently great to delay breeding 2–3 mo. in cystic cases, particularly if the cow has subsequently cycled regularly. Early pregnancy might prevent recurrent cysts.

Clapp (1) suggested that twinning may result from (a) cystic ovaries and (b) a second hereditary mechanism not involving cystic ovaries. Under this assumption, it is possible that some cows which produce twins are negative for genes causing twinning. In the present study, 188, or 71%, of the 266 twinning cows also had short estrous cycles or cystic ovaries (cystic tendency) during one or more reproductive periods. These 188 cows produced 232 (74%) of the 313 multiple births.

Twinning rates among daughters of twinning dams. If twinning only when cystic is a separate syndrome from twinning at other times, as suggested by Clapp (1), then daughters from dams twinning only when cystic should twin less frequently than daughters from noncystic twinning dams. Table 2 shows no significant differences in twinning rates expressed either as a percentage of the daughters or as a percentage of their calvings when from three types of twinning dams divided according to their history with respect to cystic tendency. Though not shown in Table 2, there was no difference in cystic tendency rate among the three groups of daughters shown, and these rates were similar to the herd average for cystic tendency.

TABLE 2

Twinning rates among daughters of cystic and noncystic twinning dams

Classification of dams	Daughters				Based on calvings of daughters			
	Total	Twinning		Expected	Total	Twinning		Expected
	(No.)	*(%)*[a]	*(S.E.)*[b]	*(%)*	*(No.)*	*(%)*[c]	*(S.E.)*[b]	*(%)*
No cystic tendency observed	62	17.7	4.8	14.7	246	6.1	1.5	4.3
Twinned only when cystic	55	16.4	5.0	14.3	216	7.4	1.8	4.2
Cystic and had twins during different reproductive periods	219	17.5	2.6	14.2	897	4.7	0.7	4.3
Total for twinning dams	336	17.4	2.1	14.3	1,359	5.4	0.6	4.3

[a] Herd average for 1,915 cows was 14%.
[b] Standard error (%).
[c] Herd average for 7,387 calvings was 4.2%.

Twin dams and sires. Only limited data were available for calving daughters from twin dams and sires. Table 3 shows twin cows had twins at $6.0 \pm 1.4\%$ of their calvings and, similarly expressed, their daughters twinned at $6.6 \pm 1.4\%$ of their calvings. The twinning rate of $3.6 \pm 1.1\%$ for 281 calvings of daughters of twin sires was lower than for daughters of twin cows ($P < 0.05$) and was not significantly different from the herd average rate of 4.2%.

Table 3 shows that the daughters of twin cows had a higher but nonsignificant twinning rate per calving ($6.6 \pm 1.4\%$) than daughters of twinning cows which were not themselves twins ($5.0 \pm 0.7\%$). The data show that twin cows are no more likely to have twins than their singly born sisters and, similarly, that their daughters do not twin at a significantly higher rate.

Service sire. Twinning rates following service of individual sires were determined and none were significantly different from the herd average rate.

Influence of sires. There were 2,076 daughter-dam pairs in this study (2) where each member of a daughter-dam pair had one or more conceptions while in the herd. Among these, there were 1,416 pairs where the daughter and the dam each had one or more calves (2). Among this latter group there were 340 daughters from twinning dams and 17.4% of these had twins, as compared with 12.0% of 1,076 daughters from nontwinning dams (2). The difference was 5.4%

TABLE 3

Twinning rates where sires and dams were twins

Group of animals	Cows	Calvings	Twins per calving		Expected rate
	(No.)		*(%)*	*(S.E.)*[a]	*(%)*
Cow was a twin	112	298	6.0	1.4	4.3
Daughters of twin cows	73	305	6.6	1.4	4.3
Daughters of twin sires	69	281	3.6	1.1	4.3
Daughters of twinning cows[b]	263	1,054	5.0	0.7	4.3

[a] Standard error (%).
[b] The dams had twins, but were not themselves a twin.

(adjusted Chi-square = 5.2; $P < 0.025$). Doubling this difference (7) yields an approximate estimate of heritability (additively genetic) of 10.8% (2).

Home-bred sires were used extensively during the study period. As a result, four major sire-families could be characterized, based on a continuous line to an original sire (Table 4). There were 23 sires, with each having at least 11 calving daughters from nontwinning dams. Table 4 shows that the observed

TABLE 4

Twinning rates (cow basis) by sire of daughters

Sire of daughters[a]			Twinning rate among daughters from: Nontwinning dams				Twinning dams	
			Rate				Rate	
		Pairs	Observed	Expected	Difference	Pairs	Observed	Expected
(group)	*(No.)*	*(No.)*	*(%)*			*(No.)*	*(%)*	
A	26–	14	14.3	19.7	− 5.4	4	0	9.8
	1–26	94	18.1	18.9	− 0.8	40	10.0	16.6
	22–1	32	3.1	12.2	− 9.1	6	0	21.5
	40–1	33	15.2	17.1	− 1.9	16	12.5	12.8
	53–40	28	7.1	11.9	− 4.2	3	33.3	18.0
B	2–	23	21.7	10.6	+11.1	11	27.3	11.6
	27–2	11	18.2	20.3	− 2.1	4	25.0	24.8
C	10–	27	22.2	8.5	+13.7	7	28.6	15.5
	35–10	112	17.0	15.7	+ 1.3	42	36.8	17.7
	41–35	81	13.6	13.0	+ 0.6	19	21.0	14.4
	42–35	46	2.2	4.3	− 2.1	13	15.4	5.7
	73–41	20	25.0	8.9	+16.1	7	14.3	13.7
D	139–137	61	24.6	18.1	+ 6.5	20	5.0	11.7
	142–137	18	38.9	20.9	+18.0	3	33.3	14.7
	6–139	21	0	6.0	− 6.0	5	0	7.7
	151–139	12	41.7	17.6	+24.1	2	0	1.3
	16–151	22	9.1	17.1	− 8.0	6	0	18.1
	5–142	83	14.4	20.8	− 6.4	33	27.3	21.5
	52–5	23	0	10.9	−10.9	18	5.6	13.7
	63–5	22	18.2	14.9	+ 3.3	7	14.3	16.3
Misc.	141–	14	28.6	10.9	+17.7	2	0	7.6
	143–	16	12.5	14.2	− 1.7	2	0	4.6
	146–	26	19.2	10.4	+ 8.8	11	0	4.8
Subtotal								
Low sires[b]						150	13.3	15.6
High sires[c]						131	22.9	13.6
Sires total		839	15.7	14.7	+ 1.0	281	17.8	14.9
Study total[d]		1,076	12.0	13.9	− 1.9	340	17.4	14.3

[a] Second sire number refers to the paternal grandsire.
[b] Observed rates lower than the expected twinning rate for daughters of nontwinning dams.
[c] Observed rate higher than the expected twinning rate for daughters of nontwinning dams.
[d] Daughter-dam pairs where each, the daughter and the dam, had at least one calving (2).

percentage of twinning daughters from nontwinning dams varied from 0 (D-6 and D-52) to 41.7% (D-151). The expected rates shown in the adjacent column of Table 4 represent the estimated percentage that should have been observed if the percentage of twinning daughters was no different for their average number of calvings than the average for the herd at a similar number of average calvings. The difference is an indication of the sire's probable contribution to the twinning tendency of his daughters. Though the dams involved in this portion of the table never produced twins, it is likely that some would have if they had remained

in the herd for a greater number of calvings. However, this factor should affect the daughters of all sires in a random manner. Because the number of daughters was low for most of the bulls, an unbiased method of grouping them was sought for the purpose of studying twinning rates among their collective daughters from another group of cows, in this case twinning dams (Table 4). Thirteen of the sires showed a decrease from the expected twinning rate and 12 showed an increase and these were respectively designated as low and high sires. As shown in the right-hand summary portion of Table 4, 13.3% of 150 daughters of low sires from twinning dams themselves had twins. This was 2.3% below the expected rate of 15.6%. The age-adjusted rate was $11.7 \pm 2.6\%$. Similarly, 22.9% of 131 daughters of high sires twinned. They were 9.3% above the expected rate of 13.6% and the age-adjusted rate was $23.3 \pm 3.7\%$. The adjusted Chi-square value for the age-adjusted twinning rates of the two groups of daughters was 4.78 ($P < 0.05$).

The last line of Table 4 shows the difference in twinning rate between daughters of nontwinning and twinning dams. The difference of 5.4%, as earlier reported (2), is significant at the 2.5% level of probability. The summary portion of Table 4 shows that twinning is heritable and that each sire and dam contribute to this tendency in the female offspring. Time trends in twinning rates, if present, may bias the interpretation of these results, since the daughters from the two types of dams (Table 4) are contemporary. However, there were no significant time trends in these data. The regression of twinning rate expressed as a percentage of the yearly calvings was 0.02% increase per year of time. The standard error of the regression coefficient was 0.04% and the standard deviation from regression was 1.4%.

General discussion. This study shows that twinning tendency in Holstein-Friesian cattle is heritable. The number of gene pairs is doubtful. No sire in the study was entirely negative when the daughters had over 50 calvings, though daughters of A-22 (159 calvings) and D-52 (137 calvings) each produced only one set of twins. The latter set was of similar sex. A reproductive characteristic such as twinning may be highly heritable, but may go essentially unrecognized because the phenotypes are inefficiently recognized. Such factors as fertilization of one egg from a double ovulation, death of one embryo but not the other, inability of some uteri to accommodate a twin pregnancy (possibly also inherited), inability of the endocrine mechanism to respond sufficiently to maintain twin pregnancies, pathogenic conditions reducing the level of physiological functioning, etc., may be the real factors contributing to our inability to recognize cows carrying gene(s) for twinning.

It is even possible to postulate that twinning tendency is primarily caused by a simple recessive character, if one cares to explore the possible causes of exceptions which do exist. For example, monozygous twinning represented an estimated 7.4% of all twin births for these cows (3), and cystic ovaries were associated with significantly more twin births at the subsequent calvings (Table 1). Other endocrine imbalances could be involved as well as unidentified environmental factors. Since genes modify or determine the biochemical path-

ways in the animal (11), different gradients of function must be frequently required to meet the demands created by other gene modifications or by varying environmental conditions. The understanding of transmission of traits involving physiological deviations specifically involves biochemical alterations and the results of these alterations may be grossly expressed in many ways, depending on other genic and environmental influences on other biochemical pathways. Thus, exact interpretation of the data presently available to animal breeders is impossible and application is quite inefficient in most cases.

Twinning rates increase with age of cow (3, 9, 12) and, even among the 266 cows that twinned, one out of four had not demonstrated the trait by the fifth calving. Because the average cow has less than three calvings, we can predict that genes for twinning are at least twice as frequent as generally recognized. Yet another factor is the high rate of twins aborted (3). It is reasonable to assume that a similar trend exists before abortions are observed. Thus, embryonic mortality may occur more frequently and could conceivably destroy one member but not the other of a twin pair.

The disadvantages of twinning are relatively minor compared with other reproductive failures. Calving expectancy was reduced for cows twinning initially at one of the first three calvings, as compared with nontwinning herdmates (3). There was no difference if twinning occurred later. Thus, one-half of the twinning cows would be involved, or slightly over two calvings per 100 for this herd. Breeding efficiency was similar to herd average subsequent to twinning for one-half of the twin births. The remainder showed variable degrees of infertility, some but not all of which would have occurred at a single birth. Thus, on a herd basis, twinning would become an economically limiting factor if about one out of ten calvings were twins.

Frequently, by the time transmission of twinning could be identified, the parents would be well established as superior transmitters of type and production and possess another important quality, longevity. From a practical standpoint, twinning rate could best be controlled by favoring older bulls showing a low percentage of twinning among the daughters as compared with their dams, or selecting sons from such bulls whose dams and their daughters have not demonstrated twinning. Even this precaution is of doubtful importance unless the herd is currently showing twinning by approximately one-fourth of the cows over 6 yr. of age.

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FACTORS INFLUENCING DAIRY CATTLE LONGEVITY

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SUMMARY

Analysis of disposal records on 505 Holstein-Friesian and 489 Jersey cattle in the USDA herds at Beltsville, Maryland, showed that 41.3% of the Holsteins and 21.3% of the Jerseys were removed from the herd as nonbreeders. Udder troubles constituted the second largest group of reasons for disposal, the percentages being 10.5 and 9.6 for the two breeds, respectively. No cattle were culled for low production or poor type in these herds during the 40-yr. period covered by the data.

Longevity of individual cows was measured in terms of age at last calving prior to disposal. The automatic selection differential for this trait averaged approximately 1½ yr. per generation for both breeds. Heritability estimates based on intra-sire daughter-dam regressions and paternal half-sib correlations did not differ significantly from zero. The correlations between longevity and first-lactation milk yield were .18 in the Holstein and .19 in the Jersey data. Similar values were obtained regarding butterfat yield.

The results indicate that differences in longevity between individual cows are determined largely by nongenetic influences. Efforts to improve this trait genetically through direct selection or by improving dairy type probably would be ineffective. Also, the study indicates that no deleterious effects on longevity need be expected by concentrating selection of progeny-tested sires on the basis of first-lactation production records of their daughters.

Longevity usually is defined as length of life. In usage regarding dairy cattle, this definition is often qualified to denote the length of the productive life. Several writers have suggested the importance of increasing longevity, both from the economic standpoint and because it affects the possibilities of improving the breed (4, 5, 9). Wilcox and Pfau (12) obtained a heritability estimate of .37 for longevity. In partial contradiction, Rendel and Robertson (11) have presented evidence from statistical data in the British Isles indicating that the average dairyman can not sacrifice emphasis in selection for total milk yield, to increase longevity.

The purpose of this study was to determine some of the environmental and genetic influences on longevity in a herd where there has been no deliberate selection among the cows for either type or production.

Source of data. The Holstein and Jersey herds used in the dairy cattle breeding experiments of the United States Department of Agriculture at Beltsville, Maryland, were the source of these data. Although the sires used in these herds were carefully selected for production characteristics, the cows were not culled deliberately at any time on the basis of production. When the increases in herd size because of excessive numbers of replacements threatened to overcrowd the facilities, the herd was reduced by eliminating sire groups without regard to their performance as individuals or as groups. The lack of selection for production among the cows in these herds is demonstrated by the small selection differential between the average production of parents, weighted by the number of daughters with records from each dam and the unweighted average production

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of the parental generations. This differential was only + 5 lb. of butterfat for the Holsteins and − 5 lb. of butterfat for the Jerseys.

Because herd reductions resulted in an arbitrary termination of a cow's life in the herd, the data on these cows have been presented only in Tables 1 and 2 and omitted from the other longevity analyses.

The Holstein data included records on all cows which had been removed from the breeding experiments since their beginning in 1919 to March 3, 1958. The Jersey data included all cows in the experiments, because the studies with this breed were terminated in 1950.

Analysis and results. Reasons for disposal. Tables 1 and 2 show the reasons for disposal in the two herds. For the purpose of this study, cows were considered as nonbreeders if they were not pregnant on completion of a 305-day lactation. Heifers were considered removed as nonbreeders if they were not pregnant when they reached 2 yr. of age. Cows reported as removed because of poor condition were those which became emaciated, would not respond to prolonged treatment, and regarding which specific diagnosis was not possible. Many of these cattle became ill shortly after calving. Cattle removed for udder troubles included those which developed one or more blind quarters after making at least one record, or had septicemia of the udder, abscessed udders, or virulent coliform and pseudomonas infections. Removals for infections were those involving kidney infections, toxic enteritis, general septicemia, pneumonia, ketosis, lung or liver abscesses, skin diseases, etc. Removals for foreign bodies included animals which died and had a definite diagnosis of hardware, or in which the diagnosed ailment had progressed to where prognosis was negative. The one undersized animal was a dwarf, being 500 lb. less in body weight at 7 yr. of age than its full sister. The animals included in the other category for the Jerseys were a few which died of poisoning, one with a broken jaw, a ruptured diaphragm, etc.

The age at disposal as reported in these studies was established as the age at the last calving prior to disposal. This age was chosen because, as previously explained, some cows were retained as much as a year or more beyond the time they would have been removed in a commercial herd. In addition to being retained for extra breedings some were retained to give them adequate opportunity to recover from an illness or injury and make a record needed for the breeding experiment. Although the cows for which disposal was delayed were few, the age of last calving was chosen as the disposal age, to prevent the data on these cows from disrupting the means for various age groups where numbers might be small. The average age of disposal as classified in these herds was 5.7 yr. in the Holstein herd and 5.4 yr. in the Jersey herd. These average ages of disposal are nearly the same as the average age of 6 yr. which is often quoted. However, these ages are 10 mo. or more less than actual, because of the use of age at last calving as the disposal age. Therefore, one may estimate that cows in the Beltsville herd remained 10 mo. to 1 yr. longer than in most herds. Much of this difference might be attributed to culling of low-producing first-calf heifers in most herds and the absence of such culling in the Beltsville herds.

TABLE 1

Reasons for disposal of cattle in Holstein herd of USDA at Beltsville, Maryland, during period from 1918 to 1958

Age at last calving prior to disposal	Total No.	Herd reductions	TB reactors	Bangs reactors	Non-breeders	Died	Poor condition	Udder trouble	Infections	Lump jaw	Broken pelvis	Ruptured vagina	Foreign body	Undersize	Slaughtered after abortion	Crippled
Heifers bred, but no terminated pregnancy	34	8	....	1	19	3	....	1	1	....	....	....	....	....	1	....
2 years	70	14	17	1	16	4	3	3	8	1	....	....	1	....	2	....
3	46	9	5	....	20	1	....	2	2	....	2	1	....	....	4	....
4	51	10	6	1	18	6	1	3	4	1	....	....	1	....	....	....
5	57	16	5	4	16	3	2	6	3	....	....	....	....	....	2	....
6	58	9	6	3	21	5	2	5	5	....	....	....	2	....	....	....
7	66	10	12	2	23	1	....	11	4	....	....	....	1	....	2	....
8	37	4	5	1	13	2	1	5	2	....	1	....	....	1	2	....
9	30	7	2	....	8	4	2	4	2	....	....	....	....	....	1	....
10	21	3	3	....	4	4	2	2	....	....	....	....	1	....	1	1
11	18	2	....	....	4	4	2	1	....	....	....	....	1	....	4	....
12	10	2	1	....	4	3	....	....	....	....	....	....	....	....	....	....
13	4	....	....	....	3	1	....	....	....	....	....	....	....	....	....	....
14	1	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....
15	2	1	....	....	....	1	....	....	....	....	....	....	....	....	....	....
Totals	505	96	62	13	169	42	15	43	31	2	3	1	7	1	19	1
Per cent of total		19.0	12.3	2.6	33.4	8.3	3.0	8.5	6.1	0.4	0.6	0.2	1.4	0.2	3.8	0.2
Per cent of total less herd reductions	409	....	15.2	3.2	41.3	10.3	3.7	10.5	7.6	0.5	0.7	0.2	1.7	0.2	4.7	0.2

TABLE 2

Reasons for disposal of cattle in Jersey herd of USDA at Beltsville, Maryland, during period from 1918 to 1950

Age at last calving prior to disposal	Total No.	Herd reductions	TB reactors	Bangs reactors	Non-breeders	Died	Poor condition	Udder trouble	Infections	Lump jaw	Severe injuries	Other	Foreign body	Bloat	Slaughtered—no reason listed	Slaughtered—after abortion
Heifers bred, but no terminated pregnancy	22	....	....	....	19	2	....	....	....	....	....	....	....	....	....	1
2 years	72	5	6	16	15	3	3	7	9	....	....	....	1	2	1	4
3	55	16	7	7	12	3	....	3	....	....	1	5	1	....	....	....
4	61	11	14	5	12	2	1	7	5	2	....	1	1	....	....	....
5	64	13	8	9	7	4	3	7	6	1	1	4	1	....	....	....
6	52	....	13	13	10	2	2	4	6	....	1	....	1	....	....	....
7	56	7	8	13	5	2	5	6	3	....	....	2	2	....	....	1
8	43	5	1	14	9	1	4	5	1	....	1	1	1	....	....	....
9	27	3	5	4	7	2	1	1	2	....	1	1	....	....	....	....
10	13	1	....	5	2	....	5	....	....	....	....	....	....	....	....	....
11	14	2	2	4	4	....	1	....	....	....	....	....	....	....	1	....
12	9	....	....	2	2	1	1	1	....	....	....	1	....	....	....	1
13	1	....	....	....	....	....	....	....	....	....	....	1	....	....	....	....
14	1	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....
15	1	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....
Totals	489	63	64	92	104	22	28	41	32	3	5	16	8	2	2	7
Per cent of total		12.9	13.1	18.8	21.3	4.5	5.7	8.4	6.6	0.6	1.0	3.3	1.6	0.4	0.4	1.4
Per cent of total less herd reductions	426	....	15.0	21.6	24.4	5.2	6.6	9.6	7.5	0.7	1.2	3.7	1.9	0.5	0.5	1.6

Automatic selection for longevity. The cows which live longer than average in a herd will usually have more calves than cows which live a shorter than average life. Therefore, the cows with the greater longevity would automatically contribute a greater number of the offspring to the succeeding generation. This should result in automatic selection for increased longevity. The extent of this can be measured by estimating the selection differential between the unweighted average longevity of the parental generation and the average longevity of the parental generation weighted according to the number of calves each cow contributed to the succeeding generation. This may be expressed by:

$$\text{Selection differential for longevity} = \frac{\Sigma(BA)}{\Sigma A} - \frac{\Sigma(BN)}{\Sigma N}$$

Where N = number of animals in each age of disposal group
A = number of terminated pregnancies in each group
B = average age at disposal for each group

In Tables 3 and 4 are shown the number of animals disposed of at the various ages in the Holstein and Jersey herds. Also shown are the number of terminated pregnancies for each age of disposal class.

The data for the foundation cattle and their outbred descendants are presented separately from the data for the descendants of homebred sires. The descendants of homebred sires were produced in a study regarding the effects of linebreeding as compared to outbreeding. However, this was considered secondary in importance to the outbred proved sire study. When budgetary restrictions

TABLE 3
Data showing automatic selection differential for longevity in USDA Holstein herd, Beltsville, Maryland

	Foundation cattle and outbred descendants			Descendants by homebred sires		
Age at disposal	No. of animals	Av. age	No. terminated pregnancies	No. of animals	Av. age	No. terminated pregnancies
(classes)		*(days)*			*(days)*	
2	36	767	22	46	729	34
3	20	1,148	34	17	1,158	33
4	28	1,457	67	13	1,460	31
5	29	1,823	89	12	1,846	41
6	47	2,192	188	2	2,279	8
7	47	2,541	233	9	2,561	46
8	29	2,891	163	4	2,901	22
9	21	3,247	134	2	3,202	13
10	17	3,617	125	1	3,804	6
11	15	4,027	124	1	4,146	8
12	8	4,366	70	....	.....	....
13	4	4,628	40	....	.....	....
14	....	.....	....	....	.....	....
15	1	5,434	11	....	.....	....
Average age at disposal *(yr.)*			6.35			3.79
Weighted average age of disposal of parental generation			7.86			5.45
Selection differential			1.51			1.66

TABLE 4

Data showing automatic selection differential for longevity in USDA Jersey herd, Beltsville, Maryland

Age at disposal	Foundation cattle and outbred descendants			Descendants by homebred sires		
	No. of animals	Av. age	No. terminated pregnancies	No. of animals	Av. age	No. terminated pregnancies
(classes)		*(days)*			*(days)*	
2	37	749	24	52	763	48
3	11	1,100	17	28	1,154	54
4	17	1,439	42	33	1,478	83
5	18	1,831	58	33	1,805	109
6	26	2,195	109	26	2,116	107
7	23	2,591	117	24	2,544	121
8	27	2,943	153	11	2,944	62
9	18	3,268	111	6	3,203	42
10	10	3,605	72	2	3,700	16
11	12	3,984	103	....	.....	....
12	9	4,355	83	....	.....	....
13	....	.....	.....	1	4,635	10
14	....	.....	....	1	5,010	8
15	1	5,349	14	....	.....	....
Average age at disposal *(yr.)*			6.30			4.61
Weighted average age of disposal of parental generation			8.08			5.88
Selection differential			1.78			1.27

forced herd reductions, these were often carried out by reducing a proportionally larger number of linebred cattle than outbreds. Evidence of this action is shown by the different average age of disposal for the two groups. Obviously, the descendants of homebred sires were removed from the herd because they had less serious ailments or injuries than the other cattle.

From these data, the automatic selection differential for longevity was found to have been 1.51 and 1.66 yr. in the two groups of Holsteins, and 1.78 and 1.27 yr. in the Jerseys. If it had been possible to deliberately select for longevity, by culling the cows as heifers on an accurate prediction of their age at disposal, it would have been necessary to have called more than 30% of each group, in order to obtain a selection differential equal to that obtained through automatic selection. This indicates that dairy cattle have been subjected to continuous natural selection for productive longevity probably over a period of more than a century.

Heritability of longevity. The heritability of longevity was estimated from within-sire daughter-dam regressions and from intraclass correlations among paternal half-sibs. The estimates are shown in Table 5. None of the regressions or correlations was significantly different from zero. Heritability of longevity as estimated from these data appears to be of such a low magnitude that selection for this characteristic would result in no appreciable improvement.

Correlation of longevity with production. There is a belief that milk and butterfat production in any one lactation are negatively associated with longevity. This belief is particularly strong in regard to the relationship of first-lactation production and longevity. To test this supposition, the within-year, within-

TABLE 5
Estimates of heritability (h^2) of longevity in USDA Holstein and Jersey herds, Beltsville, Maryland

Estimation method	Holstein			Jersey		
	No.	r or b	h^2	No.	r or b	h^2
Intra-sire daughter-dam regression	286 pairs	.004	.008	277 pairs	.025	.050
Paternal half-sib correlation	19 sire groups	.047	.188	29 sire groups	.017	.068

TABLE 6
Association[a] of first-lactation production with age at disposal in USDA Holstein and Jersey herds at Beltsville, Maryland

	Milk yield—2×,305 day, M.E.		Butterfat yield—2×,305 day, M.E.	
	r	b (days per 10 lb. milk)	r	b (days per lb. B.F.)
Holstein	.176**	.477	.195**	1.516
Jersey	.193**	.759	.179**	1.409

** Significant at 1% level.
[a] Correlations and regressions were computed within years and within systems of breeding (i.e., foundation and outbred vs. descendants of homebred sires).

system of breeding correlation between the 2×, 305-day, M.E., first-lactation records and the age of disposal of the cows was obtained. The results are shown in Table 6. It appears from these data that the correlation between first-lactation production and longevity is small but statistically significant and positive.

DISCUSSION

The main differences betwen the data presented here, on reasons for disposal, and those presented by Asdell (1), from a large number of DHIA records, are between the percentages removed for tuberculosis, brucellosis, and as nonbreeders. The high percentage of disposals for brucellosis is a result of the continuous losses from this disease up to 1927, including a serious outbreak in the herd during 1926. A serious outbreak of tuberculosis in 1935 and 1936 resulted in the high percentage of removals for this reason. Since the two outbreaks, losses from both diseases have included only sporadic reactors to annual testing. There was interest in knowing if the inclusion in the data of disposals because of these disease outbreaks might influence the results of the analysis. Therefore, the data also were analyzed regarding automatic selection, heritability, and correlation between production and longevity, with the animals omitted which had been removed for those reasons. The results were similar in all respects to the ones reported here.

The large percentage of animals removed because they were nonbreeders indicates the importance of this reason for disposal as an influence on the longevity of cows. In the Holstein herd, particularly, the percentage of removals as nonbreeders was much higher than that usually reported in DHIA data (1). This possibly was a result of the more detailed identification of reasons for

disposal in the Beltsville herds than is made in most DHIA herds. Many of the cattle in DHIA herds which are disposed of for low production are often low producers, because they have been open for a prolonged period, i.e., they were nonbreeders. Therefore, the Beltsville data may reflect more accurately than the DHIA information the importance of poor reproduction performance as an influence on longevity.

Upon casual consideration, the intensity of the automatic selection for longevity as found in these herds is surprising. However, the selection differentials are only slightly less than the differentials which can be calculated as expected, if one assumes that each cow which lives 1 yr. longer than the average will contribute one additional calf to the next generation.

It is recognized that the results in this study regarding the heritability of longevity are not in agreement with those of Wilcox *et al.* (12). Their measure of longevity was not age at last terminated pregnancy, as in this study, but number of terminated pregnancies. This might account for the difference, but the reason is not entirely clear. An additional explanation is, possibly, the fact that cattle were selected for production in the Overbrook Dairy herd, the data source for Wilcox *et al.*, and that they were not selected for production in the Beltsville herds. Selection for production would permit the higher producing cows to have more calves than low-producing cows and might also permit the daughters of high-producing cows to have more calves than daughters of low producers. Thus, the heritability value obtained in the New Jersey study might be a reflection of heritability for production and also favoritism shown to daughters of high-producing cows. The absence of selection for production in the Beltsville herds prevented these factors from contributing to the correlations and exaggerating the heritability values.

In addition to the heritability coefficients calculated directly from the data, the results in this study regarding automatic selection suggest that the heritability of longevity is low if not zero. At least two reports indicate that low heritabilities for fitness characteristics might be expected, particularly those regarding which natural selection has been prolonged and in the desired direction (2, 8). Furthermore, the importance of removal as nonbreeders as a factor influencing longevity in the Beltsville herds suggests that the heritability of this trait is low. With only one exception the heritability of fertility in dairy cows has been reported as nearly zero (2, 7, 10, 12). The low heritability found for longevity also is in accordance with the findings of Larson *et al.* (6), who reported the heritability of butterfat yield per productive day of life as being zero.

The lack of importance of genetic influences on longevity reflects directly on expectations regarding the relationship between dairy type and longevity. The evidence indicates that selecting dairy cattle for improved type will not improve longevity, because the heritability of longevity is nearly zero, and efforts to improve it through breeding in any maner are, thus, practically useless.

The low positive correlations found between longevity and first-lactation production in both the Holstein and Jersey herds at Beltsville suggest that animals which are high producers in their first lactation do not burn out or stay

in the herd for a shorter time than other cows. This finding should be of particular importance to sire selection committees when they evaluate progeny tests based primarily on first-lactation records.

In brief, these results show that longevity in the Holstein and Jersey herds at Beltsville was greatly influenced by the reproductive performance of the cows. Automatic selection for longevity is of such intensity that it would be difficult to exceed it by deliberate effort. Furthermore, the heritability of longevity appears to be so low that the effectiveness of deliberate selection for this characteristic is questionable. There is no evidence from these data that longevity is associated with low production in the first lactation. In fact, the data suggest that the higher producers have a slight tendency to remain in the herd longer than low producers, even when no selection is practiced for production.

These results, however, should not be interpreted as detracting from the importance of bringing about longevity through improved management. Disease and injury prevention practices, along with proper feeding and management, can bring about increased longevity and, therefore, improve the economic efficiency of herd operations.

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TECHNICAL NOTE

SOME OBSERVATIONS ON THE ULTRAVIOLET IRRADIATION OF MILK (CENTRIFILMER PROCESS) WITH EMPHASIS UPON ORGANOLEPTIC EFFECTS AND SPORICIDAL EFFICIENCY[1]

The belief has long prevailed that milk does not lend itself to preservation by ultraviolet irradiation, both because of the low penetrating power in this medium and because of undesirable off-flavors induced in milk by ultraviolet radiation. Somewhat at variance with this view are the rather recently published findings of Albrecht *et al.* (1), who reported that mercury resonance (ultraviolet) radiation caused virtual sterilization of raw whole milk without producing undesirable off-flavors. With the development of the Centrifilmer, it became possible for the first time to produce a smooth uniform microfilm of moving liquid which, when exposed to suitable ultraviolet radiation, greatly increases the efficiency of sterilization. This fact, plus the established usefulness of the Centrifilmer in the processing of blood plasma and in the production of polio vaccine, led us to make an exploratory study of the Centrifilmer Process as applied to the preservation of milk. The centrifugal-filmer (Centrifilmer) consists of a vertical 15-in. stainless steel bowl, the inner wall of which flares outward at a 1° angle. Suspended inside the bowl are six water-cooled ultraviolet lamps (General Electric GL816: over 90% of the total lamp output is at 2537 Å). The lamps can be operated at two intensity levels, with total irradiance at the film surface approximately 7.22×10^4 ergs cm.$^{-2}$ sec.$^{-1}$ for Level I, and 10.83×10^4 ergs cm.$^{-2}$ sec.$^{-1}$ for Level II (1.5 times Level I). Sometimes a level of about one-third of Level I was used with two lamps. In operation, fluid from the inlet container is propelled by a metering-pump through a bubble trap and flow meter into the base of the bowl which rotates at a fixed speed (1,750 r.p.m.). By centrifugal action, the liquid moves up the walls of the bowl in a micro-thin film past the radiation source and is discharged at the top in separator fashion. In our experiments, the flow-rate was 100 ml/min, film thickness approximately 15 μ, exposure time 1 sec. Detailed description of this apparatus and its operation has been given by Benesi (3) and Taylor *et al.* (4).

Organoleptic results. The machine was arranged to collect sterile samples via a closed system of rubber tubings and glass Y's. Preliminary runs showed that this system, without separating the ultraviolet lamps, imparted a rather strong off flavor to milk. This was largely eliminated by replacing the rubber with tygon tubing and collecting the samples in small Erlenmeyer flasks directly from the outlet spout of the machine. This simplified system was used for flavor experiments. Treated samples and controls were evaluated by a six-person taste panel for preference sequence. Irradiation produced intense off-flavors in milk, varying from cooked-and-burnt-cabbage, scorched, burnt-feathers, irradiated, cardboard, etc., which increased in intensity with increase in irradiation dose. However, even at the lowest level of radiation (I with two lamps) the milk was unacceptable to critical judges. Irradiated flavor was more pronounced in pasteurized skim than in pasteurized homogenized whole milk.

A series of experiments was carried out under various conditions with commercially pasteurized homogenized milk, at radiation Level I. Irradiation in air decreased the off-flavor as compared to irradiation in nitrogen. Irradiation in CO_2 at normal or pH 5.0 did not eliminate off-flavor. Deaeration for 1 hr. in a vacuum at 40° C., with a small amount of nitrogen continuously bubbling through the milk or preheating at 75° C. for 0.5 hr., alone or in combination, increased off-flavor formed by subsequent irradiation in nitrogen. Preheating also increased radiation-induced off-flavor in air. Addition of 0.01% propyl gallate, NDGA, or glyoxal (a sulphydryl protecting agent) diminished off-flavor induced by radiation in nitrogen to such an extent that these samples were superior to a control irradiated in air, which in turn was better than the control irradiated in nitrogen. A sample with 0.1% added H_2O_2 irradiated in air rated poorer than the control irradiated in nitrogen. Best results were obtained with propylgallate irradiated in nitrogen, but the flavor was deemed unacceptable by the judges; in contrast, this compound had little or no effect if the milk was irradiated in air.

Several attempts were made to remove radiation-induced off-flavor once formed. Deodorization of irradiated milk for 1 hr. in a vacuum at 40° C., with a small amount of nitrogen continuously bubbling through the milk or heating at 70° C. for 15 min., did not improve the flavor. Addition of 0.1% $NaHSO_3$ increased off-flavor. Addition of 0.01—0.1% H_2O_2 after irradiation decreased the initial off-flavor considerably after storage in a refrigerator for from one to two days but not enough to win acceptance by the judges.

Further study of the flavor reactions did not seem justified in the light of the bacteriological

[1] This study was conducted at the Western Utilization Research and Development Laboratory, Albany, California.

[2] Spores of *C. botulinum* were detoxified by heating at 75° C. for 10 min.

TABLE 1

TABLE 1

Survival of bacterial (spores) in commercially pasteurized whole milk treated with ultraviolet radiation in the Centrifilmer[a]

Date	Inoculation	Intensity level*	Before Irradiation		After irradiation (in air)		
			Total (bacteria)	Spores	Bacteria (spores) (5.5 ml. tested)	Incubated 8-ml. samples good/bad	Incubated 300-ml. sample
			(*per ml.* $\times 10^4$)				
1958							
5/17	0	I	4.0	0.017	0	8/2	...[c]
5/8	0	II	4.0	0.013	0	9/1	...
5/13	*Bacillus subtilis* (spores)	I	...[d]	1,300.0	0.0001	5/1	Spoiled
5/13	*Bacillus subtilis* (spores)	II	...	1,300.0	0.0001	6/0	Spoiled
5/13	*Bacillus subtilis* (spores)	I	...	16.0	0.015	5/1	...
5/13	*Bacillus subtilis* (spores)	II	...	16.0	0.0002	6/0	Spoiled
5/14	*Clostridium botulinum* (spores)	I	...	28.0	0	5/3[b]	...
5/14	*Clostridium botulinum* (spores)	II	...	38.0	0	9/0[c]	Spoiled
5/16	*Bacillus megatherium* (spores)	I	...	370.0	0.86	0/5	Not observed
5/16	*Bacillus megatherium* (spores)	II	...	370.0	0.58	0/5	Spoiled
5/14	*Bacillus stearothermophilus* (spores)	I	...	2,700.0	2.1	Not observed	Not observed
5/14	*Bacillus stearothermophilus* (spores)	II	...	2,700.0	1.1	Not observed	Not observed

* I. 7.22×10^4 ergs/cm.$^{-2}$/sec.$^{-1}$—total incident irradiance at film surface. II. 10.83×10^4 ergs/cm^{-2}/sec.$^{-1}$—total incident irradiance at film surface. [a] Flow rate 100 ml/min thickness of milk film approximately 15 μ, time of exposure 1 sec. [b] Spoilage caused not by *C. botulinum* but by aerobic spore-former. [c] 6-wk. observation. [d] No count. [e] No change.

results. Since radiation is relatively ineffective against many milk enzymes, radiation processing of milk would necessarily entail a supplemental heat treatment sufficient to inactivate lipase and other enzymes capable of causing deteriorative changes in the product. It was observed that heat sufficient to inactivate lipase in milk intensified the off-flavor which developed during subsequent radiation.

Microbiological results. Spores of four different bacterial species were used as test organisms: *Bacillus subtilis* (15U), representative of a type which has given much trouble in milk-distribution plants and condensaries; *Bacillus megatherium* (753), less resistant to heat than *B. subtilis*, but more resistant to ultraviolet; *Bacillus stearothermophilus* (1518), flat-sour type; *Clostridium botulinum* (62A), toxin-forming anaerobe considered to be the most radiation-resistant (gamma radiation) of all spore-forming species.

Well-washed stock spores,[2] suitably diluted and distributed in distilled water, were added to milk in Erlenmeyer flasks (capacity about two times that of volume of milk). The flasks and milk were shaken sufficiently to effect an even distribution of the spores in the milk, as judged by preliminary tests. The temperature of the milk when inoculated, and subsequently to the time it was delivered to the treatment chamber, was 10° C. or less for *C. botulinum* and *B. stearothermophilus* and 5° C. or less for *B. subtilis* and *B. megatherium*; at these temperatures, all the spores remained in the ungerminated, resistant condition up to the instant of radiation.

After irradiation, each outlet bottle connection was clamped and cut from its feed line; one of these bottles was incubated directly without opening. Eight-milliliter portions of other irradiated samples were transferred aseptically to sterile screw-capped tubes, heat-activated, and incubated with unirradiated samples; still other irradiated and control samples were heat-activated and subcultured for count, aerobically in Petri plates or anerobically in Prickett tubes. In the latter, pork-pea agar [Anderson (2)] and beef-liver infusion agar (NCA) were used. For the aerobes, the counting medium was peptone 5 g., beef extract 3 g., glucose 5 g., soluble starch 1 g., agar 15 g. per liter (pH 7.0). The samples were incubated at optimal temperature for the inoculated organism. Heat activation of the spores was carried out at temperatures ranging from 65 to 98° C., depending upon organism requirements. All of the 62A milk and subculture samples, except the 300-ml. milk bottle, were sealed with sterile sodium thioglycolate agar and mineral oil before incubation.

Spores were considered to have survived treatment if, after irradiation in milk and subculture in nutrient media, they were able to germinate and produce visible growth colonially and morphologically similar to that of unirradiated spores and if, after the treatment, irradi-

ated incubated samples underwent spoilage characteristic of the inoculated unirradiated samples similarly incubated. Results are shown in Table 1.

In uninoculated low-spore milk, radiation achieved virtual if not absolute sterility. Since the transfer of irradiated milk to many small tubes necessarily entailed some risk of contamination, an occasional spoiled sample in this (8-ml.) series may be discounted, particularly if unsupported by other evidence of survival. Although the data with *B. subtilis* are inexplicably inconsistent, it appears that an extremely small fraction of the spores survived treatment, at both intensity levels. In the single test with *C. botulinum* at a low but realistic spore level, this organism was not recovered either in the subculture medium or in incubated milk samples, although an aerobic spore-former, presumably present at the time of inoculation, was isolated. Since, in survival determinations with this organism, samples are usually observed for from 6 to 12 mo., the negative result must be accepted with some reservation. Spores of both *B. megatherium* and of *B. stearothermophilus* survived in substantial numbers at each intensity level.

The evident nonlinear relationship between spore survival and radiation intensity may be due to light-screening substances in the milk and/or to heterogeneity of the spore population in respect to radiation resistance. In an effort to shed some light upon the effect of spore clumps on the efficiency of radiation, a suspension of *B. megatherium* spores containing some large and small clumps was divided and one portion shaken vigorously for several minutes with small glass beads. Plate counts were made on both shaken and unshaken samples prior to irradiation. Although shaking of the spore inoculum increased the colony count about 28% and, as indicated by film examinations, materially reduced the size and number of clumps, this reduction in spore clumps did not appreciably increase the lethal effectiveness of the radiation.

The results reported by Albrecht (1), it should be noted, were obtained with raw uninoculated milk, presumably low in spores, comparable in this respect with our pasteurized uninoculated samples. Since, in normally produced milk, neither the kind nor number of spores can be closely controlled, irradiation of uninoculated milk does not furnish a reliable measure of sterilization effectiveness.

CONCLUSIONS

High but substerilization doses of ultraviolet irradiation (Centrifilmer Process) imparted to milk an unpleasant light-activated flavor and odor, which rendered the product unacceptable to critical consumers. Prevention of the flavor change during ultraviolet irradiation of milk, or its later elimination, poses a very difficult problem. Bacterial spores normally present in milk were not all killed by the Centrifilmer Process. Heat sufficient to inactivate lipase in milk intensified the off-flavor which developed during subsequent irradiation. The results were not sufficiently encouraging to continue attempts to produce an acceptable ultraviolet-irradiated, sterilized milk.

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INTERPRETIVE SUMMARIES OF PAPERS

RECONSTITUTABILITY OF DRIED WHOLE MILK. I. THE EFFECT ON SINKABILITY OF THE MANNER OF HANDLING FRESHLY DRIED MILK

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For many years dried whole milk has been criticized for being difficult to reconstitute in water. This was thought to be due to the physical form of the powder and to relatively large amounts of fat. Much work was done in the post-war years to improve the reconstitutability. Such variables as degree of milk concentration before drying, powder particle size, use of emulsifying agents, moisture content of powder, fat level, and others have been investigated. A solution to the problem has not been found. Recently, instantizing whole milk powder has been found to improve its reconstitutability, but it still lacks the self-wetting properties of the instant skimmilk powders.

In very recent years, several groups of workers have sought a solution by drying a milk powder with a different physical structure. One method requires that the milk concentrate be dried in films under high vacuum and at relatively low temperatures. In spite of an improved reconstitutability, this dried milk was found to lose rapidly its self-wettability after 24 to 48 hr. of storage.

The purpose of this investigation was to find a method of handling dried whole milk in such a manner that it would retain, or regain, the wettable and sinkable characteristics (both intimately related to reconstitutability) possessed immediately after drying. In addition, an explanation of observed phenomena was sought.

For the most part, the dried milk used in this investigation was film dried under high vacuum and at relatively low temperatures. To observe changes in the reconstitutability of the dried milk, a test was devised which evaluated the rate at which the powder wetted and sank into quiescent water at 25° C. These properties are jointly referred to as sinkability and the test is hereafter called the Sinkability Test.

The manner in which the dried milk was handled immediately after drying had a very significant effect on the sinkability. Crushing the milk films while the fat was still in the liquid phase resulted in a considerable loss of sinkability. However, if the films were first chilled to solidify the fat, little or no loss of sinkability resulted when the films were later crushed and handled. It was found that the initial sinkability of this dried milk could be retained if the storage temperature did not exceed 18° C.

If the dried milk was improperly handled and a significant loss of sinkability occurred, it was found that the initial sinkability, possessed immediately after removal from the drier, could be recovered and retained by a temperature conditioning treatment of the dried milk. This treatment included heating the dried milk to a temperature sufficient to melt the fat, followed by rapid chilling.

Several types of dried milk, including commercial samples, were found to respond to this treatment.

It was found that the melting point of the fat phase of the dried milk was related to the sinkability—the lower the melting point the greater was the sinkability. Dried milk containing the higher melting point glycerides responded poorly to the temperature conditioning treatment.

The phenomena observed in this investigation are attributed to the physical arrangement of the glycerides of the surface fat on the dried milk particles. If the dried milk was allowed to cool slowly after drying, or was stored at a temperature exceeding 18° C. but less than the melting temperature of the fat, a rearrangement of the glycerides in the surface fat was thought to take place. This rearrangement included the migration of the higher melting point glycerides to the air-fat interface of the surface fat. When this dried milk was placed on water at 25° C., these glycerides remained in the solid phase and presented a poorly wettable surface to the water. However, if the warm powder was chilled rapidly, a migration of glycerides was prevented and the glycerides were held in a random distribution. When this dried milk was subsequently placed on water at 25° C., there were sufficient low melting point glycerides at the air-fat interface of the surface fat to melt and present a more wettable surface to the water. Evidence that the higher melting point glycerides may migrate to the air-fat interface can be seen by holding melted butter oil at 32° C., the temperature at which sinkability is most rapidly lost. It may be noted, after several hours, that a thick layer of solidified fat forms on the surface while the fat beneath remains a liquid.

FACTORS AFFECTING THE DIRECT MICROSCOPIC CLUMP COUNT OF NONFAT DRY MILK

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In 1958, the United States Department of Agriculture adopted as a standard for nonfat dry milk a maximum Direct Microscopic Clump Count of 300 million per gram. The Direct Microscopic Clump Count (DMC) was proposed for improving the quality of nonfat dry milk (NDM). It was believed that faulty plant practices could be located and corrected by means of the DMC. It was also believed that there was a sufficiently close relationship between the DMC of the raw milk and the DMC of the nonfat dry milk to establish this standard as a reliable index of raw milk quality. It is the purpose of this paper to evaluate this criterion and to assess the suitability of the DMC as a standard for grading purposes.

The relationship between the DMC of raw skimmilk and the DMC of the NDM made from it was found to vary widely within days and seasons of the year. The DMC per gram of the NDM ranged from 1.9 to 71.0 times the DMC per milliliter of the skimmilk on 44 trials. The average increase was 9.1 times. Factors which were found to influence this relationship were: (a) The effect of break-

ing up of clumps due to agitation, (b) the removal of bacterial cells during the separation of the whole milk and subsequent clarification of the skimmilk, (c) the amount of heat treatment given the skimmilk during processing, (d) the length of time of cold storage of condensed milk prior to drying, and (e) the effect of drying. Because of the variations in the relationship between the DMC of the raw milk and the DMC of the NDM, and because of the many factors which may affect this relationship, the DMC of NDM does not appear to be a reliable method for evaluating the bacterial population of the raw milk from which the NDM was made.

THE QUINHYDRONE ELECTRODE FOR pH DETERMINATIONS IN MILK AND DAIRY PRODUCTS

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Comparisons were made of measurements of pH of milk by the quinhydrone and glass electrodes. A gold electrode cast in a plastic rod, made by the Dairyland Food Laboratory, Waukesha, Wisconsin, was used as a quinhydrone electrode when connected to a Leeds and Northrup Potentiometer No. 7655. A Leeds and Northrup Glass Electrode pH Meter No. 7664 was used for the glass electrode.

This study was divided into three phases, namely: (a) the effect on pH values of milk of the amount of quinhydrone and time between adding quinhydrone and making the reading; (b) influence on the pH values as determined by the two different types of electrodes of temperature of quinhydrone-milk mixture at time of reading, the acidity of the milk, the serum solids content, and the fat content; (c) the influence of the red color development in milk-quinhydrone mixtures.

Amount of quinhydrone and time of reading the potential. It was found upon analysis of 24 different samples of milk that both the amount of quinhydrone added and the time interval between the time of adding the quinhydrone to the milk and the time of reading the potential had a significant effect on the pH values obtained. The average pH values for additions of 5, 10, 20, and 40 mg. of quinhydrone per 10 ml. of milk were 6.646, 6.647, 6.639, and 6.636, respectively. At time intervals of 1.5, 5.0, and 10.0 min. after quinhydrone addition before reading the potential, the average pH values were 6.657, 6.640, and 6.628, respectively. The effect of the amount of quinhydrone seemed not to be significant when it was 10 mg. or less per 10 ml. of milk.

In subsequent work, the amount of quinhydrone used for each sample was constant, and exactly 1.5 min. were used between the mixing of the milk and the quinhydrone and the determination of the potential.

Influence of temperature and acidity. It was noted that increasing the temperature in the milk-quinhydrone mixture caused increasing differences in the pH values determined by the glass electrode and the quinhydrone electrode

in similar milk samples. To obtain best agreement between the two electrodes with milk samples, a temperature of about 19° C. ought to be used both in the calomel half-cell and in the milk-quinhydrone mixture.

Varying amounts of lactic acid were added to pasteurized skimmilk to give a pH range from the normal down to 4.00 as measured with the glass electrode. The pH of these samples was also measured with the quinhydrone electrode. As the pH was decreased, the difference between the value determined by the glass and the quinhydrone electrode increased, the quinhydrone value being slightly higher than that obtained with the glass electrode.

Influence of serum solids and fat content. To study the influence of serum solids content on pH values, high quality nonfat dry milk solids were dissolved in distilled water to give 1, 2, 3, . . . 20 g. of serum solids per 100 g. of reconstituted skimmilk. Then the pH values were obtained in the samples with the glass electrode and with the quinhydrone electrode. The difference in pH values obtained increased with increasing concentration, with the value obtained by the quinhydrone electrode being the lower of the two.

The difference in pH values between cream and the skimmilk from the same whole milk has been explained by a true difference in the hydrogen ion concentration. The negatively charged fat globules may tend to attract the positive hydrogen ions and thus cause actually lower pH in the cream than in the skimmilk. As a means of determining the influence of the fat content on the pH as measured by the glass and the quinhydrone electrode, samples of cream and skimmilk were mixed so that the fat content ranged from 0% (skimmilk) to 32.7%. The pH of these mixtures was measured with both electrodes. The difference between the values obtained decreased when the fat content increased. This may be due to an effect of the fat content, but may also be explained with the effect of the serum solids as determined in the earlier experiment. When the fat increases, the serum solids content decreases, thus giving a positive relation between the amount of serum solids and the difference.

Influence of color development. Investigators have observed that a red or pink color gradually develops in the milk after adding quinhydrones. They have pointed out that the potential should be determined in the mixture of quinhydrone and milk or cheese before the sample turns pink. A relationship between the drifting of the potential and the color is generally considered, but the nature of this has not been fully explained. It may be logical to suppose a reaction between quinone or hydroquinone and one or more of the milk constituents in such a way that the same reaction results in both a red color and in a drifting of the potential.

By use of a Hunter Color and Color Difference Meter it was possible to study some of the factors that influence the red color development in milk-quinhydrone mixtures. Generally, the factors which caused increasing difference between the pH values observed by the glass electrode and the quinhydrone electrode also caused increasing redness in milk-quinhydrone mixtures. There is, therefore, apparently a relationship between the color reaction and the pH value obtained with the quinhydrone electrode.

If the development of the reddish color in the milk-quinhydrone mixture might be due to some reaction between free reactive groups in the protein and the quinone or the hydroquinone, it may be prevented by the addition of formaldehyde to the milk before the quinhydrone addition. The results for ten samples showed that the addition of formaldehyde caused a significant decrease in the development of the reddish color. The effect of formaldehyde, however, need not be due only to the tying up of reactive groups in the protein. The binding of basic groups will cause lower pH values in the milk and for that reason also prevent the color development.

Conclusions. The protein and fat content, which have been shown to have some influence on the pH difference (pH glass electrode—pH quinhydrone electrode), undoubtedly have small significance in skim or whole milk because of the small variations in these factors. In cream, the pH obtained with the quinhydrone electrode probably will agree better with the pH obtained with the glass electrode than in whole milk samples at the same temperature. In dairy products with high protein content, the quinhydrone electrode probably will show lower pH values than the glass electrode.

Increasing serum solids, increasing temperature, and increasing pH caused increasing red color in the milk-quinhydrone mixture. These results may be explained by a possible reaction between the quinone or hydroquinone and the proteins.

STUDIES ON PSYCHROPHILIC BACTERIA. I. DISTRIBUTION IN STORED COMMERCIAL DAIRY PRODUCTS

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Expanding applications of refrigerated storage and transport in the dairy industry emphasize the importance in product deterioration of those bacteria capable of relatively rapid growth at low temperatures. Such bacteria are given the name psychrophiles. To clarify their role in spoilage, as well as to devise better means of predicting and combatting spoilage, more information is needed about the relative frequency of occurrence of specific types of psychrophilic bacteria in commercial dairy products and about their biochemical activities. Fundamental studies of the nature and activities of psychrophiles have been few and conflicting.

In this investigation, a large number of fresh samples of commercially packaged milk, chocolate drink, cream and cottage cheese were secured from nine dairy plants and stored at 40° F. for 1 wk. to permit selective growth of psychrophilic bacteria. The predominant microbial types in each sample were then identified and studied.

Except for a few yeasts, the dominant psychrophilic microorganisms were all gram-negative bacilli. The over-all frequency of occurrence of microbial types was: 70.6% *Pseudomonas*, 7.9% *Alcaligenes*, 9.2% *Achromobacter*, 0.7% *Flavo-*

bacterium, 10.8% coliform bacteria, and 0.8% yeasts. This distribution was quite similar among the dairy products, and showed little seasonal variation.

It appears that under strictly controlled conditions of low temperature storage a limited number of psychrophilic types are able to multiply sufficiently rapidly to become quantitatively important in dairy products. Additional studies of the type and extent of breakdown of milk constituents accomplished by these bacterial types may be of help in further defining the problem of spoilage of refrigerated dairy products.

STUDIES ON PSYCHROPHILIC BACTERIA. II. PSYCHROPHILIC COLIFORM BACTERIA IN STORED COMMERCIAL DAIRY PRODUCTS

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The presence of coliform bacteria in dairy products has long been recognized as a valuable indicator of post-pasteurization contamination and as a potential contributor to spoilage. Although microorganisms of this group grow over a wide temperature range, little is known about their minimum temperature for growth. The opinion is commonly held that coliforms may occur sporadically in the psychrophilic microflora, but are relatively unimportant in food deterioration during refrigerated storage.

In a previous paper, the authors reported that coliform bacteria comprised 10.8% of the psychrophilic cultures isolated from commercial fluid dairy products and cottage cheese after storage at 40° F. for 1 wk. The ability of coliform bacteria to multiply sufficiently rapidly under these conditions to attain a population of millions per milliliter, and their apparently frequent incidence in dairy products, raises anew the question of their importance in refrigerated spoilage.

It was the purpose of this investigation to characterize in greater detail these coliform cultures and to classify them, where possible, according to species.

Of those cultures which produced the typical rapid acid fermentation of lactose, four were classified as *Escherichia intermedia*, five as *Aerobacter cloacae*, 13 as *Aerobacter aerogenes*, and three remained unidentified. The largest group, comprising 36 cultures, were markedly atypical of coliform bacteria and were classified as aberrant forms of *A. cloacae*.

Noteworthy from the standpoint of food spoilage potential was the ability of all cultures except those of *A. aerogenes* to attack protein, and the ability of most of the cultures to break down butterfat. The aberrant *A. cloacae* cultures, however, were almost totally unable to ferment lactose, and most of the other cultures produced no gas in the process.

Such atypical coliform bacteria, if they occur frequently in dairy products, present a problem in laboratory identification. Because of their lack of gas production from lactose, they would be missed in coliform tests based on this character.

It is suggested that psychrophilic coliform bacteria may occur more frequently than has been commonly recognized. They may frequently be misclassified because of their atypical biochemical activities, or, because of their rapid growth at 98.6° F. also, they may not be recognized as psychrophiles.

SOME OBSERVATIONS ON THE ULTRAVIOLET IRRADIATION OF MILK (CENTRIFILMER PROCESS) WITH EMPHASIS UPON ORGANOLEPTIC EFFECTS AND SPORICIDAL EFFICIENCY

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Sterilization of milk without appreciable change in its fresh-milk properties has been a long-sought goal of the dairy industry. Frequent experimental efforts have been made to utilize irradiation as a substitute for heat in milk sterilization. Ultraviolet, unlike gamma irradiation, has received limited consideration because of its low penetrating power in milk and because of undesirable off-flavors imparted to milk by ultra-violet light. The development of the Centrifilmer made it possible for the first time to produce a smooth, extremely thin moving film of uniform thickness which, when exposed to a suitable ultraviolet source, increases the efficiency of sterilizatioin.

It was the purpose of this study to explore the possible usefulness of the Centrifilmer Process as a means of sterilizing milk. Irradiation with the Centrifilmer (ultraviolet light) rendered the milk unpalatable to critical consumers. Attempts, by physical or chemical means, to prevent the development of off-flavor or to eliminate it after irradiation were not successful. Resistant bacterial spores added to milk before irradiation were not all killed. The results were considered not sufficiently encouraging to warrant further efforts to produce an acceptable ultraviolet-sterilized milk.

CRITICAL FEATURES OF GOOD DAIRY FEEDING EXPERIMENTS[1]

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After an investigator decides on the kinds of cows to use in a feeding experiment, on the diets to be compared, and on the measures of performance to make, he is confronted with the problem of designing and running the experiment in the most efficient manner possible.

It is impossible to feed different diets under exactly the same conditions. Either the different diets must be fed to different cows at the same time or to the same cows at different times. Even when all cows are fed the same diet continuously, milk production and other items of interest will vary between

[1] The original paper is in the Journal of Dairy Sci., 43: 193. 1960.

cows and from time to time on the same cow. Furthermore, the cows will differ in the way their behavior changes with time; e.g., some will fall relatively rapidly in milk production and others relatively slowly. Thus, a part of the difference in behavior seen when feeding different diets is not attributable to differences in the diets but to natural differences between cows or to nondietary things which change with time, such as weather and stage of lactation. The latter sources of variation introduce an element of uncertainty into the interpretation of experimental results, and their effects are called experimental error. In good experiments, the researcher tries to allot the cows to the diets and to conduct the experiments in such a way that experimental error is made as small as possible. Furthermore, good experiments are set up so that an estimate of error size can be obtained from the experimental data. To conclude that diets are of different value is a good bet only if the observed differences in behavior are sufficiently large compared to experimental error.

The general principles to be followed to obtain small experimental error and to permit estimation of error can be found in books on Applied Statistics under the topic Experimental Design. These expositions usually include the arithmetic necessary to estimate error. Effective use of the principles of error control in dairy feeding work requires recognition of the factors which contribute to variation in milk production and other items of behavior. Also, feasible means of taking these factors into account must be recognized.

Suppose two diets are to be compared, and 16 cows are available. A fair (unbiased) comparison can be obtained by allotting eight cows to each diet in a completely random fashion. Although fair, the comparison is subject to large error, because the production ability within a group of cows ordinarily varies widely. The coefficient of variation or error per cow is usually about 25%. Since the error for comparing two diets is given by the formula, $C\sqrt{2/n}$, where C is the coefficient of variation and n is the number of cows on each diet, the error in the supposed experiment is about 12%. Standard practice requires that a difference of about twice the error of a dietary comparison be realized in order to make it a safe bet (odds of 19:1) that the two diets are different in their effects on milk production. An error of 12% is unacceptable, for differences of 2 to 4% are of practical importance. However, the error of a dietary comparison can be reduced, without increasing the number of cows, by reducing the error per cow.

Cows which are high (or low) producers in early lactation tend to remain high (or low) producers throughout lactation, and cows producing side by side show similar time changes in production because of similar time changes in environment. This similarity of behavior is particularly noticeable if the cows are in the period between peak-lactation and mid-gestation. These two items—the tendency of cows to maintain their rank in production level, and the tendency for the time changes in production to be the same for cows in the same environment—are the basis for error control.

Continuous trials form one class of dairy feeding experiments. In these, all cows to be used are observed for 2 or 3 wk. on a standard diet. They then are

allotted to the experimental diets and remain on the latter until the close of the experiment. The major portion of the variation between cows is removed from experimental error by taking into account the production on the standard diet. Sometimes the cows are divided up in such a way that the groups on the different diets had essentially the same average standardization performance—a procedure called balancing. Sometimes, however, the cows are allotted to diet at random. In either case the standardization performance is used in some way when interpreting the data, and experimental errors per cow of 7 to 11% can be realized for the measures on milk. Little better can be done with identical twins.

Change-over trials are another type of experiment. In this type, several experimental periods, each several weeks in length, are used, and a given cow receives different diets in the different periods. There are the cyclic, round-robin, or Latin-square types in which the diets fed a given cow are all different, and there are the switch-back or reversal types in which a diet received by a given cow in an early period is fed to it again in a later period. Simple examples of change-over trials are:

Latin-square							Switch-back		
	Treatments received by cow group							Treatments received by cow group	
Period	1	2	3	4	5	6	Period	1	2
1	A	B	C	A	B	C	1	A	B
2	B	C	A	C	A	B	2	B	A
3	C	A	B	B	C	A	3	A	B

Augmentation of these simple patterns provides change-over designs which permit the comparison of more than two or three diets. Small errors are obtained because diets are compared on the same cow, thus eliminating cow differences as a source of error, and at the same times, thus eliminating effects common to all cows of factors which vary with time.

Experimental errors per cow per period vary considerably, but they average 5 to 6% for the usual production measures in the Latin-square type of trial. Errors are even lower in the switch-back trials, averaging 2 to 3%. These low errors come about because of the return in a late period to a diet fed in an early period—a situation which permits an accounting for more of the between-cow variation in time trend than is possible in the other types of trials.

In change-over trials performance of a given cow on a given diet can be affected by the diets fed the cow in previous periods. These effects are called carry-over or residual effects. Ramifications of the Latin-square type designs and accompanying arithmetic procedures are available which permit the researcher to estimate the carry-over effects and thus more properly interpret results. Carry-over effects can not be handled well with switch-back trials, but the low errors of these trials make them valuable in a research program, particularly if carry-over effects are not large.

HIGH LEVELS OF GRASS SILAGE FOR MILK PRODUCTION WITH NO GRAIN, MEDIUM, AND HIGH GRAIN FEEDING. I. INTAKE, MILK PRODUCTION, AND BODY WEIGHT CHANGES

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In many parts of the country, where weather conditions interfere with making hay at the most desirable stage of maturity, grass silage is the appropriate alternative to hay. Furthermore, as the nutritive qualities of forages stored for feeding dairy cows continue to improve, it is important to learn more specifically about the milk producing qualities of forage. It was the purpose of this study to determine how much milk could be produced when grass silage was the main source of nutrients and also to determine how the cows would respond on such a ration to feeding varying amounts of concentrates. Most studies on this subject in the past have covered only parts of a lactation or were not balanced with respect to some important factors. It was considered important to determine what would happen over much longer periods of time and so the study covered three lactation periods on the same group of 18 Holstein and six Guernsey cows.

Three levels of grain feeding were used, no grain, medium grain (at the rate of 1 lb. per 6 lb. 4% milk), and high grain (at the rate of 1 lb. per 3 lb. 4% milk). Cows were fed only 5 lb. alfalfa hay but all the grass silage they would eat when offered four times daily. Pasture was supplied during the summer.

The ability of the cows to produce milk differed from cow to cow and one of the most important results of the study was that it showed so clearly how important this ability was in determining what the cow would do with grain. The potential producing ability of a cow was estimated to be the average of all her previous 8-mo. age-corrected records adjusted to a 4% milk basis. Eight-month records were used to get away from differing effects of pregnancy when some cows failed to conceive on schedule.

There was a decrease in the amount of roughage the cows ate as the amount of grain increased—about ¼ lb. decrease in roughage intake for each 1 lb. grain eaten. But it was also found that within a breed the low potential cows dropped more in roughage intake when fed grain than the high potential cows. On the all-roughage ration cows ate 2.40 lb. hay equivalent per 100 lb. body weight, on the medium grain ration cows ate 2.14 lb., and on the high grain ration, 1.91 lb.

The average 4% milk production on roughage alone of four groups of cows with an average potential of 8,800 lb. was 6,500 lb. in 8 mo., but four groups with an average potential of 10,900 lb. gave 8,000 lb. On high grain these lower potential cows increased 2,400 lb. milk, but the higher potential cows gave 4,900 lb. more milk. There was no carry-over from one lactation to the next of the effect of any level of feeding.

There was also a close relationship between the ability of the cow and its efficiency in making milk from grain. The lowest potential Holstein group (average of 8,900 lb. potential) gave .58 lb. milk for each pound of grain, but the highest producing group (11,700 lb. potential) gave 1.35 lb. milk for each

pound of grain fed. With the high producers the change from medium to high grain gave just as much increase in milk per pound of grain as the change from no grain to medium grain, but with the low producers the change from medium to high gave slightly less milk per pound grain than the change from no grain to medium grain.

The main differences in lactation curves appeared in the first 6 wk.—on no grain the cows dropped right from the first week, on medium grain they held even for 4 wk. then dropped, and on high grain they peaked at 4 to 6 wk. as expected, and then dropped. After 6 wk. the slope of the curves was about the same for all groups on a proportional basis.

When the cows went on pasture there was a much greater response by the cows on no grain than by the cows receiving grain. There was also a greater response by cows late in lactation than by those in early lactation.

Loss in body weight from the first to fourth months was 106 lb. for cows getting no grain, whereas the high-grain cows were gaining an average of 16 lb. After that time the no-grain cows gained much more rapidly than the high-grain cows but they never caught up with them.

The results of this experiment show very clearly the importance of the high producing ability of the cow—its appetite for roughage was apparently better, its actual production was much higher, and it produced more milk from a pound of grain than the low producer, showing that it was more efficient. The amount which cows would produce on roughage alone, when the roughage consisted mostly of grass silage, also seemed to depend to some extent on its producing ability. Though the cows in this study did produce about as much on an all-roughage ration as the average cow in this country produces with some grain, in most of the eight groups it would have been uneconomical not to feed some grain, since the cost of grain was less than the value of the extra milk produced.

EFFECT OF RAPID GROWTH WITH FATTENING OF DAIRY HEIFERS ON THEIR LACTATIONAL ABILITY

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In normal rearing, dairy heifers are fed only a small amount of grain. Their growth rate may be greatly accelerated by increasing the energy content of the ration through grain feeding. Such rapidly grown heifers may reach the normal 24-mo. body weight at 20 mo. or less. If rapidly grown heifers produced as efficiently at any given body weight as slower growing ones, the extra cost of raising them might be recovered by earlier lactation. Some previous experiments have indicated, however, that rapidly grown heifers will not milk well; so the cost of raising, as well as the poor milk yields, makes them very uneconomical.

This investigation was made to test the effects of rapid growth on lactation by using identical-twin heifers. Identical twins are expected to produce alike if reared and treated alike. Differences within the pairs after different rearing

should be mostly due to the treatment and not to differences in genetic milking ability.

A total of seven pairs of twin calves was used. One of each pair was fed normally, with only roughage after 10 to 12 mo. of age. The mate was fed roughage plus all of a dairy feed mix (concentrates) which it would consume readily after 6 mo. of age. The latter feeding resulted in fattening and rapid growth. At 2 yr. of age the fattened twins weighed 899 lb., compared to 683 lb. for the normals, a difference of 32%. At calving, the fattened ones averaged 964 lb. in weight, compared to 727 lb. for the normals.

After calving, all heifers were fed adequately for good production, and the twins of each pair were fed the same daily ration of concentrates; therefore, production differences were not affected by different feeding in lactation. The previously fattened twins failed to yield as much milk as the normal-lean ones in six out of seven pairs. In the full first lactation the fattened heifers produced 85% as much FCM as the normals. The average lactation of the fattened heifers was 20 days shorter than the normals. Five pairs were also compared in their second lactations. Again, the normally reared heifers averaged higher FCM yields than the fattened ones, 8% more, even though they had been fed and handled the same since their first calving.

In searching for reasons for the poorer production of the rapid-growth heifers, their udders were examined after the second lactation. Four out of five pairs which were sectioned had noticeable deficiencies of development of milk secreting tissue in the outer edges of the glands. This probably accounted for part of the low production of the fattened heifers; but it is also postulated that possibly differing metabolism effects during the lactation enabled the leaner heifers to turn their feed into milk more efficiently than could the fattened heifers.

The results of this study are considered significant because they confirm some previous observations indicating that fattening growing dairy heifers may depress lcatation, and because this observation has been made with identical-twin heifers.

COMPARISON OF SIX- AND TEN-DAY COLLECTION PERIODS FOR DIGESTION TRIALS WITH DAIRY HEIFERS

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Digestion trials are expensive and time-consuming and, as a result, several substitute methods have been suggested to replace the total-collection procedure. Historically, the time of collection periods has been 14 to 21 days. During the past 15 yr., ten to 14-day collection periods have been consistently used. More recently, several publications have been released in which five- to seven-day collection periods were used. The only published work studying short periods was a seven-

day collection trial with beef animals. This study was initiated to compare six- to ten-day collection periods with dairy heifers.

Eighteen animal-digestion trials were conducted with a basic ration of oat hay. Coefficients of correlation for all nutrients except the ether extract fraction between the six- and ten-day trials were significant at the 1% level of probability. When the differences between the values obtained at six and ten days were analyzed by means of the "t" test, none was found to be significant at the 5% level of probability. The study shows that there was little advantage in extending the collection periods from six to ten days. As the collection perods are shortened, it would be well to consider lengthening the preliminary periods to at least 18 days.

EFFECT OF INITIAL LEVEL OF MILK PRODUCTION ON RESPONSE TO FORAGES OF VARYING QUALITY

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Research with dairy cows is both expensive and time-consuming. Despite the need for an abundance of information on the feeding value of pastures, hays, and silages, it is not economically possible to feed all possible forages under all possible feeding conditions. For these reasons, there is need for information which will be helpful in extending the range of application of available data. A frequently asked question concerns the application of data obtained with cows producing 25 lb. of milk per day to herds averaging 40 lb. per day.

Data obtained from feeding trials with dairy cows on pasture or with silage fed free choice were used to study the influence of level of production on response to the ration. In all cases, grain was fed on the grain : milk ratio system. The results of this study indicate that under the conditions of the experiments, a pasture capable of sustaining normal milk production in cows producing 20 lb. of milk per day can reasonably be expected to do equally well with cows producing 40-50 lb. per day when grain is rationed on a grain : milk ratio basis. Although there was a tendency for the higher producing cows to be more adversely influenced by low quality silage, the differences in response due to initial milk production were not statistically significant.

INHERITANCE OF TWINNING IN A HERD OF HOLSTEIN-FRIESIAN CATTLE

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Hereditary control of multiple births in swine, sheep, and goats has been well established. However in cows, twin births are comparatively rare. One can

not be certain of the twinning rate for some breeds of cattle because an insufficient number of complete breeding herd records have been studied. We are reasonably certain that there is considerable variation between the several breeds and even more certain that some herds run much higher than other herds. These findings suggest but do not prove that twinning in cattle is hereditary.

Twinning is not a desirable characteristic in dairy cattle and might well be considered as abnormal when it occurs. Most recognize that about nine of every ten heifers born with bulls are sterile (free martins). Also, abortions and dead calves are much higher for twin births. Further, nearly one-half of all twin births are accompanied by retained afterbirth. Complications after calving twins, often leading to difficult breeding, occur more frequently than following single births. Production is generally lower, perhaps due to the added stress of bearing twins.

It was the purpose of this study to extend our knowledge about twinning with a view towards its control by selection.

In this study, 14% of the cows had twins at one or more calvings. Out of the 7,387 total calvings by these cows, 4.24% of them were twins. Heifers had twins about one-fourth as often as mature cows.

If cows conceived within 75 days after having cystic follicles on the ovaries, twins were produced at 15% of the subsequent calvings. After 75 days the rate was nearly the same as the herd average. Daughters from the cystic cows had twins no more or no less frequently than if their dams had had twins in the absence of cystic ovaries. These findings show that cystic ovaries are associated with increased chances for twinning by the cow having them. Withholding breeding for 75 days after cysts are observed, to reduce the chances of twinning, would be a questionable practice, since early pregnancy may well prevent recurrent cysts.

Twin cows are no more likely to have twins than their singly-born sisters. Daughters from twin cows or twin bulls are a little more likely to have twins than daughters from singly-born cows which had twins.

Both the cow and the bull significantly contribute to the tendency of their daughters to have twins. Five per cent more daughters from twinning cows have twins than daughters from nontwinning cows. Sires were grouped into high and low on the basis of the twining rate of their daughters from nontwinning mates. When from twinning dams, 13.3% of the daughters of low bulls and 23.3% of the daughters of high bulls had twins.

Selection against twinning does not appear practical, at least for most herd owners. The average cow has less than three calvings and relatively few would have twins by this time. Older cows have proven their worth as producers and not infrequently as desirable brood cows. A herd bull would be quite old and generally quite desirable in many ways before the breeder would be aware that its daughters have an undesirably high twinning rate. This is the only presently known way to determine twinning rate, since at service the bull has no influence on whether its mate has twins.

Certain precautions do appear practical. One should be able to reduce a high twinning rate or to control present levels in a herd by favoring older bulls showing a low percentage of twinning among the daughters as compared with their dams. Selecting herd sires whose immediate relatives have a low rate of twinning would also improve the chances of not using too many bulls transmitting twinning tendency to their daughters. Even these precautions are of questionable importance unless about one-fourth of cows in the herd over 6 yr. of age have had twins. Economically speaking, type, production, and longevity are of much greater importance; therefore, efforts to decrease twinning rate should not interfere with improvement of these traits.

FACTORS INFLUENCING DAIRY CATTLE LONGEVITY

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At what age does a cow leave the herd? As a rule, at about 6 yr., when the cow stops producing or breeding, unless death is caused by disease, infection, broken bones, or from eating too many nuts and bolts.

Would it be profitable to concentrate on efforts to lengthen a cow's life? Some think it may have possibilities for improving the herd. A longer life would theoretically mean more calves and more milk.

But a study of 40-yr. records at the Dairy Cattle Research Branch in Beltsville, Maryland, shows that automatic selection for long life—incidental to selection for high production and other qualities—is already so intense that it would be practically impossible to exceed it by deliberate effort. Moreover, the heritability of long life appears to be so low that the effectiveness of deliberate selection for this trait is questionable.

The evaluations were made to determine environmental and genetic influences on long life in nearly 1,000 Holstein and Jersey cattle. None of the herds had ever been culled for low production or por type during the 40 yr.

Reasons for disposing of cows are important in judging over-all herd longevity. Evaluation of the Beltsville herd disposal records showed cows were removed for various diseases, infections, and udder troubles. A large number, however, were removed as nonbreeders—41% of the Holsteins and 24% of the Jerseys. This indicates the importance of nonbreeding as an influence of life expectancy.

The large percentage of nonbreeders removed may have resulted because more detailed disposal records are kept in the Beltsville herds than are made in most Dairy Herd Improvement Association herds. The Beltsville data may reflect more accurately the importance of poor reproduction as an influence on longevity than DHIA information.

A positive relationship—low but significant—was found between long life and first-lactation production in both Holstein and Jersey herds. This suggests that cows that are high producers in their first lactation do not burn out or stay in the herd for a shorter time than other cows. In fact, the data indicate that

the higher producers tend to stay in the herd longer than low producers, even when no selection is practiced for production. This finding should be of special significance to sire-selection committees when they evaluate progeny tests that are based primarily on first-lactation records.

The lack of importance of genetic influences on longevity reflects directly on expectations regarding the relationship between dairy type and longevity. The evidence indicates that selecting dairy cattle for improved type will not improve longevity, because the heritability of longevity is nearly zero and efforts to improve it through selection in any manner are, thus, practically useless.

These evaluations do not detract from the importance of bringing about longer life through improved management, say the scientists. Disease and injury prevention practices and proper feeding and management can bring longer life and improve efficiency of herd operations.

OUR INDUSTRY TODAY

THE DAIRY INDUSTRY IN A CHANGING WORLD[1]

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It is a real privilege for me to give the keynote address at this 45th Anniversary meeting of the National Dairy Council. I think it is extremely valuable in any group to survey what has been done—especially, if you make this survey in light of changing demands and changing situations.

During the past 2 or 3 yr. I have been happy to take part in the celebration of the 75th Anniversary of two Agricultural Experiment Stations, one at Ohio and one at Wisconsin. In both cases I pointed out that when I first started teaching agricultural chemistry, I spent some time reviewing for the students the early development of the experiment stations from Rothamsted, England, down through the various stations in this country. However, today we seem to be so busy teaching the citric acid cycle and the very latest developments in nutrition, that we have little time for historical developments.

I also pointed out that I felt it was important for all of us to emphasize the contributions that agricultural experiment stations have made in this country. They have had a tremendous impact on research and development in many areas of human activity. With the success of the federal support for agricultural experiment stations, we have today increasing support in many areas, both in the medical sciences and in the basic sciences. There is no better example of the combination of basic and applied research than that conducted in our agricultural experiment stations throughout the country.

Some of you may wonder why I emphasize agricultural experiment stations on this occasion. I do so for several reasons. First, during my long association with the Wisconsin Agricultural Experiment Station, I found that there was a close relationship with the National Dairy Council. While the National Dairy Council does not cover so broad a territory as an experiment station, it has been interested in applying basic knowledge to applied problems—and in carrying the conclusions of such studies to those who need and are interested in the results.

Secondly, specifically in the field of nutrition, the two groups have grown together and during the past 40 or 45 yr. we have seen tremendous developments in the field of nutrition, and much of this development has centered around milk in one way or another.

Thirdly, while the agricultural experiment stations have been supported both by federal government and local governments, and the National Dairy Council has been supported through voluntary contributions within the industry, both groups have recognized the fact that this support would be forthcoming only if dividends were received.

So, on this occasion, we should first recognize the leaders who had the vision to organize the National Dairy Council, to secure adequate support for it, and to use this support so that further funds would be forthcoming. In some of the publications of the National Dairy Council, members have been called shareholders, and I am inclined to believe that these shareholders have gained more from their investment in this organization than almost any organization in which they have invested their funds.

But I am sure you did not invite me here today to pass out bouquets on what has been done. More important is to survey past contributions in light of the present situation and what changes need to be made so that the shareholders of the future will be guaranteed their dividends. To do this, we should remind ourselves of the over-all objective of the National Dairy Council program: To promote optimum health and human welfare through adequate use of milk and its products in accord with scientific recommendations, and thus contribute to a more secure American agriculture and national well being.

When the Dairy Council was organized in 1915, we were just beginning to see the development of a new field of human endeavor—the science of nutrition. It was during this period that we first heard the word protective food. The terminology was actually initiated by Professor McCollum when he was at the University of Wisconsin. He spoke of milk as a protective food because it protected against ophthalmia. Today we know that the factor involved was vitamin A and that milk is a very rich source of this vitamin.

Milk was protective for many other reasons—because of its calcium content, its high quality protein, and the vitamin which later became known as riboflavin. In fact, the promotion of optimum health through adequate use of milk was a natural and it was just impossible not to reach this objective of the Council. It was easy to transfer some of these facts to the public through pictures of experimental animals. The title came to be known Milk Made the Difference and recently the Dairy Council has remade some of these pic-

[1] Address given January 25, 1960. Forty-fifth Anniversary Meeting of the National Dairy Council at Chicago, Illinois.

tures and they tell the same story they did 40 yr. ago.

Today, we like to use the term wholesome food rather than protective food. A wholesome food is a product which is sound, clean, fit for human consumption, free of any objectionable qualities, and which makes a significant contribution to human dietary requirements.

We have introduced here a new emphasis and that is the fact that a food makes a significant contribution to human dietary requirements. This immediately raises the question—What are these requirements? The development of this information has been tremendously interesting to me, since I have had the privilege of following a great many of the studies. I could spend several hours this morning describing some of these details to you.

May I mention just three or four examples, to emphasize the important part that milk has played in basic experimentation? In the 1920's, anemia was prevalent in growing children and farmers were experiencing a serious malady in young pigs which came to be known as thumps. Through experimentation with milk diets, it was possible to produce an anemia in experimental animals which was not corrected with iron salts alone but responded only when copper also was added.

This finding did much to help eliminate the anemia in children and it solved an important agricultural problem. However, more significant was the fact that when we added iron, copper, and also manganese to milk, it was possible to grow mammals on milk alone through several generations. This demonstrated for the first time that milk was a complete food in all respects except two or three minor traces of mineral elements.

Another example: After it had been clearly demonstrated that nicotinic acid or niacin was the anti-pellagra factor and that it functioned effectively in human cases of pellagra, some difficulty arose when methods were developed for determining the amount of nicotinic acid in common foods. It was soon found that those foods which had prevented pellagra, such as meat and certain vegetables, were high in this vitamin, and those which allowed the development of pellagra such as corn, were low in nicotinic acid. However, one serious discrepancy was soon observed; namely, that milk was extremely low in this vitamin, and yet Dr. Goldberger had used milk in the treatment of human pellagra. In fact, milk was so low in this vitamin that we wondered how infants living almost exclusively on milk could survive and not develop pellagra. After several years' work, it was found that the explanation was related to the fact that the amino acid, tryptophan, can function as nicotinic acid in the animal body. Thus, milk was an effective food because it supplied adequate quantities of tryptophan and, therefore, the need for niacin was not as great.

Milk also played an important role in elucidating the final facts in the vitamin B_{12} story. For many years we tried to produce pernicious anemia in the experimental rat; in fact, we had high hopes, because we finally reached a highly purified diet which we were sure contained no anti-pernicious anemia factor. We had used milk diets in an attempt to produce a cobalt deficiency in the rat. Milk contained very small quantities of cobalt, but even so, a deficiency was not produced. Today we know that the small amount of cobalt present was there as vitamin B_{12}, and only very small quantities of this vitamin are needed. All of our purified diets containing casein supplied enough vitamin B_{12} because of the tiny quantities present even in highly purified casein.

Today we know that milk, especially cow's milk, carries small quantities of vitamin B_{12}, but adequate to supply the needs of all mammals. It is true that the milk from different species varies somewhat and it has been possible to produce pernicious anemia when goat's milk alone is fed.

We can not complete this story without emphasizing that the dairy cow produces vitamin B_{12} within its own digestive tract, if adequate cobalt is present in the diet. Since most of the food consumed by the cow is devoid of vitamin B_{12}, we certainly have been fortunate that nature provided this mechanism for producing vitamin B_{12} not only in the milk but also in the liver of these animals which a few decades ago was used as the only source for the treatment of pernicious anemia.

It is perhaps unnecessary to mention the early studies on vitamin D milk, except for the fact that today we take this product for granted and give little or no consideration to rickets in our children. I merely want to add that the development of the basic findings and the methods of producing and distributing vitamin D milk challenged many workers and took the cooperation of many people.

During this brief story I have alluded to the fact that from these studies it became possible to determine the daily requirements for specific nutrients and to develop methods for measuring the amount in each food. The actual daily requirement for a human may vary from 200 to 300 g. of carbohydrate to 50 to 75 g. of protein, down to 1 g. of calcium per day. Even the vitamins that are needed in the so-called trace amounts vary greatly in the quantity of need. For example, vitamin D is needed in the amount of a few thousandths of a milligram—while vitamin C is needed to the extent of 75 mg. per day. This is a variation of over a thousandfold.

All of this information has become available during the past 45 yr., and has been tabulated by the Food and Nutrition Board of the National Research Council. Fairly complete tables were published in 1943 and revisions were made in '45, '48, '53 and, most recently,

1958. The figures are given as recommended dietary allowances and when compared with those used in many under-developed countries are considered quite generous. However, in a country of plenty, there seems to be no reason to be skimpy in our recommendations. Thus, anyone can consult these tables and find the suggested intake for most of the better known nutrients and for individuals of different age groups and of different activities and so on.

These values would be of little significance if we did not know the amount of each nutrient in all of our available edible foods. It took somewhat longer to tabulate these data, since routine analysis, after methods have been perfected, are not too interesting to most research workers, and it remained for the impetus during the war to finally develop the tables of food composition. Again, tables are now available and, with a little arithmetic, one can calculate the intake of any or all of the individual nutrients which any one of us takes in in a day or a week.

These, then, are the results of thousands of man-hours of diligent research in the field of nutrition. And the National Dairy Council has, through its research grants, contributed in many areas which have helped facilitate these final results. But perhaps more important is the fact that the Council pioneered in the development of methods to carry this scientific information to the public. And in this connection I can not help repeating the statement I made several years ago:

". . . The practical value of every social invention or material discovery depends upon its being adequately interpreted to the masses. The future of scientific progress depends as much on the interpretive mind as it does upon the creative mind.

"The interpreter stands between the layman, whose knowledge of all things is indefinite—and the scientist whose knowledge about one thing is authoritative. The scientist advances knowledge. The interpreter advances progress. History affords abundant evidence that civilization has advanced in direct ratio to the efficiency with which the thought of the thinkers has been translated into the language of masses."

The Council has been very effective in this translation.

But the results of research bring about change. You have already indicated that we are living in a changing world. So, with all this knowledge of the nutritional requirements and the composition of all foods, it is possible today, if necessary and if we wish to do so, to build diets for humans and farm animals which are practically devoid of milk and milk products. However, if anyone were to suggest this, I think we might have greater furor than that now brought about by attempts to subsidize milk production or to help the farmer secure adequate income for his labors. There would be tremendous criticism of the dairy farmers today if we did not have adequate milk products to meet the demand. However, at present, the reverse is true. The national milk production at the present time is somewhat over 126,000,000 lb. per year. Fortunately for our farmers, the production per cow has increased and the efficiency of production has also moved upward. Although the production of milk has increased, our population has increased more rapidly and, therefore, the per capita consumption of 700 lb. is considerably less than the pre-war levels of 850 lb.

Let us look at this figure a little more closely. The average of the two figures is equivalent to about a quart of milk per day for everyone in this country. Now we realize that this is unevenly distributed, both as far as age groups are concerned and as far as geographic distribution is concerned. We also know that this includes all dairy products and, therefore, a certain part of the quart is consumed as ice cream, cheese, and other dairy products. But, from the nutritional point of view, this quart supplies the entire calcium requirement, most of the phosphorus requirement, adequate intake of riboflavin, almost sufficient vitamin A, as well as a number of the other nutrients. Or, we may look at it another way. If we wanted to live on nothing but milk, by fortifying it with a few minerals I mentioned earlier, an individual of normal activity could get along very nicely on 4 qt. of milk per day. The reason for this larger intake is due to the fact that 4 qt. are needed to supply the energy requirement.

It has been estimated that today it requires 7 min. of labor to purchase 1 qt. of milk. Or, if we lived on 4 qt., it would require a little less than 0.5 hr. of work. In other words, through milk, each of us could secure our daily requirements in a very acceptable and attractive form by working half an hour each day.

This emphasizes two points. One is the great nutritional value of milk and dairy products, which many of us have emphasized for years. The other is that today's products are being produced and sold at very low cost. This is the point where I would like to stop and talk about the future.

The dairy industry has contributed to optimum health and human welfare. It has done so by making milk available where needed through educational and promotional procedures. It has not only emphasized the nutritional qualities, but it has improved sanitary procedures so that milk today is a safe and sound food to consume. In other words, the dairy industry has contributed both to the elimination of deficiency diseases and infective diseases. Indirectly, it has helped improve health throughout the world, through the use of dry skimmilk powder.

It is unnecessary for me to go into the details here, but the low cost of skimmilk powder has

improved the quality of many foods in this country, and has had a tremendous influence on the improvement of health in other parts of the world. The fact that the surplus of skimmilk powder has been reduced sufficiently so that the United States Department of Agriculture is no longer making this product available, has thrown many difficulties in the way of continuing the promotion of health, especially among children and certain underprivileged countries.

Where you have fallen down, if I may say so, is on the latter part of your objective ". . . and thus contribute to a more secure American agriculture." Why should the dairy farmer make available this highly nutritious food at this exceedingly low price? It is time that the price of a quart of milk or the price of corresponding milk products is increased to a point where it is commensurate with the nutritional contribution which this food makes.

I know that I am now getting into economics and that I am not an economist, but this is an area in which the Dairy Council and related groups need to work. I believe I can make this statement with some confidence, since many of us who contributed to the early development of our knowledge of nutrition were not medical men or clinical workers; yet, through the laboratory, methods were found to eliminate many of the deficiency diseases. Therefore, some of us non-economists may be bold enough to suggest that a similar approach should be used to increase the price of milk so that we may have a more secure American agriculture. And, as I indicated previously, there would be a great furor in this country if our agriculture were not secure enough to produce sufficient milk to meet the demand for milk products.

Now some of you may say that it was a laboratory worker—one Dr. S. M. Babcock, who put us on the wrong track as far as estimating the value of milk is concerned. However, I am sure that Dr. Babcock would laugh at us today if he knew we were still using fat to measure the nutritive value of milk, in light of more modern developments.

To be sure, there are many other new frontiers in research relating to the dairy industry. I shall mention a few of them. But again, I must say that they are insignificant in relation to the basic problem of securing an adequate price for high quality, wholesome, nutritious food. There are many studies to modify liquid milk so it may be stored for longer periods of time and shipped over greater distances. We have dehydrated milk products that are easily reconstituted, and there will undoubtedly be many further developments in this area. We have triple-concentrated milk and we are now begining to hear about canned sterile cream, which may help distribution greatly.

To settle some of these problems, we need more basic studies in the physical and biochemical properties of the constituents of milk —how temperature, salt concentration, and other factors affect these ingredients. Progress has been made because of great advances in the field of biochemistry, physical chemistry, and bacteriology. Further progress will depend upon greater knowledge concerning the proteins, the lipids, and even the sugars in milk. I hope these studies will continue and, as new findings are made, new bold experimentation will be conducted.

Another new approach which I believe should have greater interest is the production of foods containing dairy products. I believe we have carried the tradition of identifying each individual milk item far beyond its usefulness. We have federal laws identifying butter and in the past we may have had reasons for some of these regulations. However, from a nutritional, and from a dietetic, point of view, there is no need for identifying dairy products as they are originally produced. Perhaps the public is not ready to accept this idea, but certainly I would hope that the research worker would tackle some of these problems and perhaps from the results will come the new products which will enhance the use of dairy products.

I suppose we will have to continue research to combat the criticism of milk products in certain types of disease, especially cardiovascular difficulties, arthritis, and so on. I believe we should not run scared, but on the other hand we must know the facts.

There is no question but that some people have over-emphasized the danger of animal fats. While I do not profess to know all the facts, I think one can summarize in very simple language—and that is, if one consumes a mixed diet, with ordinary amounts of milk products in the diet, there can be no danger except in certain individual cases that are especially prone to some of these effects. Certain individuals may have more severe arthritis if their calcium intake is high. In these cases, one certainly would not want to recommend high quantities of milk or milk products which carry calcium. However, the next speakers will cover these questions in greater detail.

Yes, great progress has been made and the future looks hopeful if we approach some of these problems from a basic point of view. All I can do is to emphasize that milk is a nutritious product. It is a product which may be used in many different forms which are highly acceptable to the American public. We need to look upon it in the form of a 1960 model rather than a 1915 model.

Congratulations on what the National Dairy Council has done and I have great assurance that it will make equal progress in each of the coming decades!

OUR ASSOCIATION

AMERICAN CHEMICAL SOCIETY AWARD IN THE CHEMISTRY OF MILK

The Canvassing Committee of the American Chemical Society for this award for the coming year hopes that many worthy members of the American Dairy Science Association will be nominated for the Award in the Chemistry of Milk. Nominations do not have to be prepared for research workers nominated last year.

This award is popularly known as the Borden Award, because it is sponsored by the Borden Company Foundation, Inc. Its purpose is: "To recognize and encourage outstanding research achievements in the chemistry of milk in the United States and Canada." The award consists of $1,000 and a gold medal. An allowance of $150 is provided for travelling expenses to the meeting at which the award will be presented. A nominee must be a resident of the United States or Canada and have accomplished outstanding research in the chemistry of milk and dairy products or in fundamental research that contributes directly and materially to the knowledge of milk and dairy products.

The essential features of preparing a nomination are as follows:

Any member of the Society (except a member of the Award Committee) may submit one nomination for each award. Such proposal must be accompanied by a biographical sketch of the nominee (including date of birth), a list of his publications and patents, specific identification of the work on which the nomination is based, and an evaluation and appraisal of the nominee's accomplishments, particularly the work to be recognized by the proposed award.

The evaluation and appraisal of the nominee's accomplishments is of great importance and should be prepared with care. It provides an opportunity to spotlight contributions of particular significance to theory or practice, and to show how these have been applied. An analysis of patents is especially valuable. If the nominee is not the sole author of important publications listed or evaluated, his contribution should be specified.

Eight copies of this material must be furnished for distribution to the members of the Award Committee. The nomination and its accompanying material must be received in the Office of the Executive Secretary of the American Chemical Society before June 1 of the year preceding that in which the award is to be made.

G. R. Cowgill
Stuart Patton
S. R. Hoover, Chairman
Eastern Utilization Research and Development Division, USDA,
Washington 25, D.C.

ABSTRACTS OF PAPERS PRESENTED AT MEETING OF SOUTHERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

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MANUFACTURING SECTION

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Status of manufacturing milk in the South. J. C. Flake, Evaporated Milk Association, Chicago, Illinois.

Agricultural leaders and others are striving to develop a balanced agriculture in the South and to raise the economic level of the family sized farm operator. The small or medium sized dairy herd is important to this agricultural program.

In the older and more successful dairy sections, there is a good balance between the utilization of milk for fluid products and for manufactured dairy products. In the South, a great deal of interest has developed in production of milk for manufacturing, although production per farm has remained rather low.

The importance of manufacturing milk to the South is illustrated by the fact that there are many times as many manufacturing milk shippers in the region as there are fluid milk producers. It is estimated that some of the southern states have as many as 30,000 manufacturing milk shippers.

While thousands of the individual dairymen are doing an excellent job and are making substantial incomes, an appraisal of the current situation reveals the need for much more progress than has been accomplished to date in the following: (1) Convincing manufacturing milk dairymen to consider the enterprise as a major part of their farm business rather than as a minor sideline. (2) Emphasis by agricultural leaders and others on manufacturing milk production. (3) Increase in size of herds, production per cow, and income per herd. (4) Improvement in feed production and herd management practices. (5) Employment of labor saving facilities and equipment. (6) Raising enough well-bred heifers for herd replacement and expansion. (7) Convincing young people to consider dairying as an integral part of their farm operation. (8) Reversing the significant trend toward decreasing numbers of producers and volume of milk in many areas.

Most of the technical problems leading to this situation have been solved. The necessary information is available, as are labor-saving facilities and equipment at reasonable cost, to enable the farmer to increase volume and efficiency to profitable levels. An urgent need is to obtain more rapid application of these facilities and wider adoption of improved feed and management practices.

Making a business out of manufacturing milk production. G. S. PARSONS, North Carolina State College, Raleigh.

In the last few years, many dairymen throughout the country have made a business out of the production of milk for manufacturing purposes. With only a small investment, they have increased their production, produced a higher quality milk and, at the same time, made their jobs easier, and more pleasant, and more profitable. The question is—How has this been possible? They have done this by changing to labor-saving devices in dairying—such as elevated stall milking parlors, cow-to-can milking machines, mechanical coolers, self feeding of silage from horizontal silos, loose housing of dairy cattle, and ground level hay storage.

By the use of these labor-saving devices, the manufacturing milk producer has taken more pride and interest in this enterprise. He milks more and higher producing cows, feeds more and high quality roughages, uses artificial breeding, uses available family labor, and follows good management practices. These are the practices producers must follow in order to make a business out of manufacturing milk production.

To better acquaint you with these labor-saving devices, how they are built, advantages of each, and results from their use, slides will be used.

It is obvious we will have farmers producing milk for manufacturing purposes for many years. Why not help these dairymen make a business out of this enterprise? This would be an answer to many of the manufacturing milk problems facing us today.

If we take advantage of our time, face up to our responsibility, we as agricultural workers can sell and show dairymen everywhere how they will be able to do less work, yet produce more milk and increase their profits, by using these labor-saving devices in dairying.

We all share this responsibility. Each of you has a job of helping to make sure that the new ideas of today become the farm practices of tomorrow.

The manufacturing industry and reasons for the current trends. M. F. ELLMORE, Virginia Polytechnic Institute, Blacksburg.

A study of the situation has revealed a downward trend in the volume of milk for manufacture delivered to southern plants. The problem facing processors is lowered operating efficiency due to fractional utilization of plant facilities. A large and important segment of the industry is affected. An understanding of the causes responsible for the situation is a prerequisite to the development of plans to solve the problem.

There is one basic reason for the current trends. Farmers are selecting other alternative uses for their production resources. Examples of alternatives which appear more attractive are: off-farm employment, other agricultural production, social security, etc.

Failure of the industry to adjust to changing conditions has resulted in loss of the favorable position once held by manufacturing milk production. Educational agencies can teach production practices designed to improve the efficiency and reduce drudgery in existing production units. This is a stopgap measure. Education alone will not encourage enough new producers to balance the normal turnover.

It is incumbent upon the processing segment of the industry to realize the competitive position of production. If volume of deliveries is to be maintained, or increased, a favorable economic position for this enterprise must be restored.

Advantages of manufacturing milk to Southern agriculture. G. M. BASTIN, University of Kentucky, Lexington.

The trend to larger, more specialized dairy units in the past few years causes one to think of what might happen to the entire dairy picture in the South. There is no doubt that there will continue to be changes. In the glamour and excitement of new and expanded programs, we often overlook some sound and economical projects in our community.

Production of milk for manufacturing purpose has some advantages that are often over-

looked. The South is blessed with market outlets for manufacturing milk. According to AMS-301, March, 1959, there are 146 butter plants, 79 cheese plants, 91 condensed and evaporated plants, 41 plants making nonfat dry milk for human food, and three plants making dry whole milk.

Milk produced for manufacturing purposes provides a market for small dairymen at a minimum equipment cost. It provides an income that might not be realized otherwise. It gives an individual the opportunity to grow into the dairy business by shifting from cream production to milk production. Manufacturing milk production is also a logical level to start dairying. Another advantage of manufacturing milk production is the excellent field work provided by most plants. Milk plants provide employment with a payroll that is advantageous to a community, and most personnel connected with these plants provide excellent agricultural leadership.

A rapid method for determining milk protein. W. K. STONE, L. F. CREMERS, AND N. R. THOMPSON, Virginia Agricultural Experiment Station, Blacksburg.

A consistent amount of protein nitrogen was released by carefully controlled steam-distillation of milk made strongly alkaline with NaOH. When using a vacuum-jacketed, double expansion bulb distillation flask, a distillation head with platinum gauze filters, and a Hopkins-type condenser, the amount of steam-distilled ammonium nitrogen was proportional to the amount of protein in the milk. Ten grams of milk, 10 ml. of 30% NaOH, and 10 ml. of 10% $BaCl_2$ solution were added to the distillation flask and steam-distilled for 10 min. The ammonium nitrogen was received in boric acid containing methyl red–methylene blue indicator. Titration was with (ca. 0.01 *N*) standard HCl.

When raw milks of different protein content were analyzed for steam-distilled and Kjeldahl nitrogen, a linear relationship was found. The mean steam-distilled nitrogen was 5.53 mg. per 10 g. milk. The standard error of estimate for a single determination was $\pm$ 0.08. Accuracy of per cent total protein determined by the steam-distillation method was of the same order as for per cent protein by the Kjeldahl method. The reagents and apparatus were inexpensive. Determinations were made rapidly, and the method appeared applicable for large-scale routine testing of milk for total protein.

Determining protein in milk by the steam distillation method of Kofranyi. C. VANDERZANT, M. A. BROWN, AND I. W. RUPEL, Texas Agricultural Experiment Station, College Station.

Protein determinations were made at 2-wk. intervals on (a) milks from individual animals in several herds, (b) bulk milk deliveries from individual farms to a milk processing plant, and (c) bulk milk delivered to processing plants in various parts of Texas. The method employed was a modification of the steam distillation procedure of Kofranyi. A sample of milk (10 ml.) to which are added equal quantities of 10 *N* NaOH and 10% $BaCl_2$ is steam-distilled for 9 min. The ammonia is received in 3% H_3BO_3 to which a few drops of Tashiro indicator are added. The ammonia distilled over was titrated with 0.05 *N* HCl. A conversion factor was established by comparing results obtained with the modified Kofranyi method with those obtained with the official Kjeldahl procedure. The steam distillation method for determining protein in milk proved as accurate as the Kjeldahl method. Little or no difference was observed in the value of the conversion factor if milks were used (a) from animals of one breed only, (b) mixed herd milk, and (c) late lactation milks.

A colorimetric method for determining protein in milk with Buffalo Black. C. VANDERZANT AND W. R. TENNISON, Texas Agricultural Experiment Station, College Station.

The dye solution was prepared by adding 600 mg. of Buffalo Black per liter of buffer composed of 980 ml. citric acid solution 21 g. per liter, and 20 ml. 0.2 *M* $Na_2HPO_4 \cdot 7\,H_2O$. The pH of this solution was 2.35. To 0.5 ml. of milk in plastic centrifuge tubes was added 20 ml. of dye solution. This mixture was stirred for 5 sec. by an automatic stirrer and the insoluble protein-dye complex was separated by centrifugation for 10 min. at 1,500 g. The concentration of the unbound dye in the clear supernatant liquid was determined at 575 mμ in a Spectronic 20 Colorimeter. A linear relationship was observed when the light absorption values were plotted against the protein concentrations as determined by the official Kjeldahl procedure. Increasing the period of mixing of dye and milk from 5 sec. to 2 hr., with or without shaking, did not change the light absorption values. With this method, no difficulties were experienced in removing the supernatant fluid from the protein dye complex. Filtration through paper removed a small amount of dye from the filtrate.

Influence on pasteurized milk flavor of rancid milk in the raw supply. A. V. MOORE, A & M College of Texas, College Station.

Commercial pasteurized milks, in some markets are flavor-characterized as astringent, coconut, and rancid. The incidence of these flavors appears to have increased as the problem of rancid raw milk supplies has become greater. Both rancid and nonrancid raw milks were pasteurized. To the latter, 5, 10, 15, and 20% levels of rancid milk were added immediately before pasteurization, and flavors of these products determined daily for six days. In only one of nine trials could

rancid flavor be detected at the 20% level immediately before pasteurization. No consistent sequence of off-flavor development occurred as the samples aged, but coconut and rancid characterized practically all samples after four days, if 5% or more rancid milk had been added to nonrancid milk.

Influence of rate of cooling upon the development of hydrolytic rancidity in milk. E. R. JARMAN, K. L. NEELEY, A. V. MOORE, AND J. J. WILLINGHAM, Texas Agricultural Experiment Station and Texas Technological College, Lubbock.

Eighteen sets of milk samples were analyzed to determine the influence of rate of cooling upon the development of hydrolytic rancidity. The samples were collected from eight cows and composited. Tests were made immediately after collecting to determine the extent of free fatty acids in the fresh milk. The samples were then divided into equal lots, with one lot being cooled to 38° F. within 3 min. by immersion in a brine bath. The other sample was cooled slowly by setting in a 38° F. refrigerator. By using approximately 300 ml. of milk, the temperature was down to 38° F. within 1 hr., plus or minus 10 min. Tests were run after the first 1 hr. plus cooling and again 12 hr. later. The same samples were warmed to 80° F. and again tested to determine the increase in the free fatty acid degree. Results of these trials will be presented.

The inflence of sugars upon lipolysis in milk. B. J. DEMOTT, University of Tennessee, Knoxville.

The inhibiting effect of formaldehyde upon lipolysis and the reaction between lysine and milk sugar raised the question as to the possibility of a reaction between milk sugar and the enzyme lipase.

A portion of mixtures containing raw skimmilk, phosphate buffer at pH 6.3, and formaldehyde or sugars was heated to 145° F. for 30 min. and the second portion stored at 50° F. for 30 min. Both portions were then mixed with equal quantities of pasteurized homogenized milk and incubated at 104° F. for 1 hr. The free fat acidity was then determined by the method of Frankel and Tarassuk. Formaldehyde was found to reduce the lipolysis to about one-half the value of the samples to which no inhibitor was added. Sugars had a slight inhibitory effect upon lipolysis, lactose and galactose being more effective in lipolysis inhibition than sucrose or dextrose.

Species of lipolytic anaerobic spore-forming bacteria found in milk and cream. B. M. MCCLURE AND E. R. GARRISON, University of Arkansas, Fayetteville.

Only limited information is available as to the species of *Clostridium* that may be present in milk and cream, and the lipolytic ability of these anaerobes is virtually unknown. In this investigation, samples of milk and cream were obtained periodically over a 2-yr. period from the producers' cans delivered at a manufacturing milk plant and four cream-buying stations. Ten milliliters of each product were placed in sterile tubes containing 3 ml. of Vaspar and heated in a water bath at 85° C. for 15 min., then cooled in water to 37° C. and incubated at this temperature for ten days. All samples that showed gas fermentation, and most of the samples that showed proteolysis but no gas, were plated on modified Sugiyama medium to which Nile blue sulphate and a fat emulsion were added. The plates were incubated 72 hr. at 37° C. in an anaerobic incubator filled with nitrogen.

When lipolytic colonies were present, a transfer from one colony from each milk and cream sample was purified and studied. With few exceptions, lipolytic organisms were obtained only from samples that showed gas fermentation. Only 69 (5.5%) of the 1,251 cream samples produced gas and 32 (2.6%) yielded lipolytic organisms. Gas fermentation occurred in 1,090 (23.8%) of the 4,375 milk samples and 558 (12.9%) gave lipolytic cultures. The incidence of samples that showed gas formation was much higher during the winter than during the summer months. There was also a close positive correlation between the sediment content of the milk and the percentage of samples that produced gas. Of 200 cultures studied, 154 were classified as follows: *Clostridium bifermentans* (27), *Clostridium caproicum* (33), *Clostridium septicum* (11), and *Clostridium sporogenes* (83).

Some studies of the free-fat acidities in milk. W. W. OVERCAST, J. D. SKEAN, AND DOUGLAS H. STAMPER, University of Tennessee, Knoxville.

The increased use of pipeline milkers and the bulk system for handling of milk has resulted in increased agitation of the raw milk. Much of this agitation occurs before the milk is cooled and activation of the milk lipase occurs. A survey was made of the acid degree values of pasteurized homogenized milk from 13 plants, using the method of Thomas *et al.* The acid degree values ranged from 0.75 to 2.68, with a median of 0.88. Three of 26 samples were criticized as rancid. According to the usual interpretation of these acid degree values, all of the samples were at least at the threshold of rancidity. In addition to this survey of milk from plants, the season of the year, stage of lactation, and types of feed as they influence the free-fat acidities in herd milk with bulk handling have been studied.

Flavor problems in rearranged milk fat. J. B. MICKLE, Oklahoma Agricultural Experiment Station, Stillwater.

Previous work (J. Dairy Sci., 42: 389. 1959.) demonstrated that the molecular structure of milk fat could be changed using a directed interesterification reaction. The butter made from this rearranged milk fat was significantly softer than butter made from normal milk fat. One of the problems associated with rearranging milk fat was the rancid, metallic-like flavor which often developed. This paper reports data obtained in a study concerned with this flavor and a method of counteracting it.

Refining milk fat after it had been rearranged appeared to remove the flavor encountered in this work. The refining process consisted of neutralizing the free fatty acids in the milk fat, then bubbling steam through the fat under vacuum. Conducting the interesterification reaction in a nitrogen atmosphere, rather than in air, appeared to improve the flavor of the resulting fat. Severe heat treatments appeared to intensify the flavor. The latter finding was in agreement with reports in the literature.

Radiostrontium in dairy products. B. J. DeMOTT, D. G. EASTERLY, AND R. G. CRAGLE, University of Tennessee, Knoxville, and U.T.-A.E.C. Agricultural Research Laboratory, Oak Ridge, Tennessee.

Milk from four Jersey cows given single oral doses of Sr^{89} and Ca^{45} were separated in a DeLaval Junior Model separator. Concentrations of the isotopes were determined by counting the oxalate precipitates. The cream showed concentrations about one-half that found in the milk and the skimmilk showed concentrations slightly greater than the whole milk.

Cheddar cheese made from milk of dosed cows, as well as from milk to which the isotopes were added, showed a concentration of Sr^{89}, on a weight basis, of about five times that of the milk from which it was made. The concentration of Sr^{89} in relation to Ca^{45} was slightly higher in the curd than in the whey. Calculated on the basis of literature value protein contents, Cheddar cheese contained only about 80% as much Sr^{89} as did the milk from which it was made.

Phosphate reactivation in pasteurized milk. J. B. MICKLE, R. DIAB, AND H. C. OLSON, Oklahoma Agricultural Experiment Station, Stillwater.

Previous work from this station (J. Dairy Sci., 41: 708. 1958) indicated that there was an increase in the phosphatase activity of milk during incubation after that milk had been laboratory pasteurized at 144.5 or 152.4° F. The objectives of this study were to determine the effect of: (a) incubation times and temperatures and (b) overheating the milk during pasteurization, upon changes in the phosphatase activity of that milk during incubation. After pasteurization, the milk was incubated at 52, 72, or 90° F. Portions of it were tested for phosphatase activity after one, two, and four days of incubation.

The data indicated that the phosphatase activity of the milk stored at 72 and 90° F. increased faster during incubation than did the activity of similar milk stored at 52° F. The activity of samples stored at all three temperatures, however, continued to increase as the time of incubation increased. Standard plate counts of milk samples stored at 72° F. ranged from nine to 280 thousand, four to 23 million, and 12 to 30 million after one, two, and four days of incubation, respectively. Overheating the milk during pasteurization appeared to reduce the rate of increase in phosphatase activity during subsequent incubation.

De Laval ultra-high temperature pasteurizer. T. B. HARRISON, University of Tennessee, Knoxville.

In August of 1959, a DeLaval Ultra-High Temperature Pasteurizer was installed. Flavor difficulties were encountered, so the vat method was again used until the steam controls were changed. Recirculation of the milk through the flow diversion valve was stopped and this reduced the cooked flavor of the milk.

At the filler the milk had a pronounced cooked flavor. After 15 hr. at 38° F., the cooked flavor had almost disappeared.

The chloride content of milk. W. A. KRIENKE, Florida Agricultural Experiment Station, Gainesville.

The complementary relationship that exists between lactose and chlorides in milk is important in evaluating S.N.F. A change (up) of 0.01% chloride is reflected by a change (down) of 0.17% lactose, resulting in SNF being down by 0.16%. Because of this, all values for SNF of milk should be accompanied by corresponding chloride values, or the SNF values should be adjusted on the basis of a standard chloride value and so reported. Chloride values were obtained on daily samples of milk from 11 cows covering periods of 6 to 7 mo. and on six consecutive daily samples of milk from 160 cows. Fat and lactometer determinations also were made on each sample.

In general, there were only slight daily changes in the chloride content of milk of each cow. For several cows there was no change or only a slight increase in chloride content at the end of the lactation period.

Individuality of the cows appears to be the greatest factor concerned with the chloride content of milk. Some cows in their fourth and fifth lactations produced milk of very low chloride content and some in their first lactation produced milk of constantly high chloride content.

It is suggested that mixed milk from many cows should not exceed 0.12 to 0.13% chloride content.

Sanitary aspects of welded lines for milk processing. H. C. Olson and J. B. Mickle, Oklahoma State University, Stillwater.

A study was made of the sanitary condition of the equipment and the sanitary quality of the milk in a plant using welded milk lines instead of the conventional gasketed sanitary connections. The sanitary condition of the lines was evaluated by visual inspection, swab tests, rinse tests, and line tests on the pasteurized milk during passage through the equipment. The sanitary quality of the finished product was evaluated by standard plate counts and coliform counts on random samples at each inspection and on hourly samples run by the plant personnel during each day's operation.

The results indicate that there was no excessive contamination or build-up of milk stone in the welded lines used in conjunction with an automatic CIP system. The bacterial counts on the milk in the plant and on samples delivered to several cities indicate that the milk was of satisfactory sanitary quality. The counts on the milk from the plant under observation compared very favorably with those on similar plants operating in the same area. It may be concluded that welded milk lines used in conjunction with an automatic CIP system are satisfactory for the processing of Grade A milk.

Consumer preferences for cottage cheese. H. C. Olson and Dorothy Strozier, Oklahoma State University, Stillwater.

A survey was made of consumer preferences for salt content, rate of creaming, size of curd particles, and acidity (or pH) of cottage cheese. Additional information was gained by interviewing shoppers in food stores regarding their opinions on cottage cheese currently marketed.

The results indicated that a salt content of 1.0 to 1.25% was preferred over higher or lower concentrations. With the cheese used in these trials, a creaming rate of 30% had the highest preference rating. A medium-sized curd (cut with ⅜-in. knives) was preferred over large curd (⅝-in. knives) or small curd (¼-in. knives). The data on preferences for acidity (pH) showed a rather wide range, with cheese high in acid (pH about 5.0) preferred over low acid cheese (pH about 5.2).

The consumers' opinions of cottage cheese indicate a rather high regard for this product. The major comments favoring cottage cheese were that it is low in calories, high in food value, particularly proteins and minerals, inexpensive, easy to serve, requires no special preparation, and adds milk constituents to the diet. The principal objections were that it has poor keeping quality and lacks flavor.

A survey of cottage cheese manufacturing operations in Alabama. R. Y. Cannon, Alabama Agricultural Experiment Station, Auburn.

Eleven plants producing cottage cheese in Alabama were observed for manufacturing operations, and samples were collected for observations of keeping quality. Organoleptic and bacteriological analyses were made on the samples originally and at weekly intervals during storage at 5 and 10° C.

Variations in manufacturing operations and finished product include: (a) Cooking temperatures ranged from 120 to 132° F.; (b) curd temperature after cooking ranged from 37 to 75° F.; (c) seven of the plants chlorinated the final wash water with chlorine content ranging from 4 to 25 p.p.m.; (d) butterfat content ranged from 2.83 to 4.88%, with six of the samples being below 4% butterfat; (e) moisture content ranged from 76.07 to 83.18%, with two samples being above 80%; and (f) drainage as determined 24 hr. after creaming ranged from 15.7 to 34.26%.

The samples obtained 24 hr. after packaging had an average flavor score of 39.45, average pH of 5.25, and average SPC and psychrophilic counts of 90,700 and 138,500, respectively. After storage at 5° C. for 1, 2, and 3 wk., average flavor scores were 37.36, 36.20, and 31.73, respectively; average pH's were 5.16, 5.06, and 4.95, respectively; average SPC's were 1.8, 31.5, and 65.4 million, respectively; and average psychrophilic counts were 1.4, 23.7, and 93.1 million, respectively. After storage at 10° C. for 1, 2, and 3 wk., average flavor scores were 34.82, 32.45, and 28.09, respectively; average pH's were 5.09, 4.85, and 4.77, respectively; average SPC's were 5.6, 82.1, and 85.3 million, respectively; and average psychrophilic counts were 5.1, 33.1, and 59.2, respectively.

Rate of inoculum of cottage cheese and its influence on yield and manufacturing time. J. N. Morgan, E. R. Jarman, and J. J. Willingham, Texas Technological College, Lubbock.

A total of 32 vats of cottage cheese was made, using inoculum rates of 5, 10, 15, and 20%, with eight vats of each rate. The average time from setting to cutting for the 5, 10, 15, and 20% inoculations was 5 hr., 20 min.; 3 hr., 47 min.; 3 hr., 21 min.; and 3 hr., 5 min. There was no significant difference between the yields obtained at the various rates of inoculation. The whey losses from the various lots were approximately the same.

It is suggested from these trials that a considerable saving in time, plus added protection from failures caused by phage attacks, could be accomplished by using higher rates of inoculation than is commonly practiced.

Use of frozen ripened lactic cultures in the manufacture of cottage cheese. J. H. Martin and J. T. Cardwell, Mississippi State University, State College.

A ripened lactic culture which had proven reliable by starter activity tests was frozen in 16-oz. containers at −20° F. The contents of one of these containers was transferred directly into 10 gal. of bulk starter milk which was used in making the cottage cheese. An additional supply of culture was refrozen about every 60 days from a can of bulk starter.

The study covered a period of 1 yr. and involved 36, 50-gal. batches of cottage cheese made from low heat NFDM manufactured in the Mississippi area. The make procedure as recommended by Angevine was followed.

The results show that the frozen culture remained equally effective in its ability to produce the necessary acid in cottage cheese after being frozen and refrozen periodically for one year. The average time from setting to cutting was 282 ± 18 min. The whey acidity at cutting, moisture content of the finished curd, yield (corrected to 80% moisture), and body score averaged $0.61 \pm .019$, $80.9 \pm 1.6\%$, 102.5 ± 4.5 lb., and 3.7 ± 0.4, respectively, for the entire experiment. These figures compared favorably with those from a previous study involving cultures transferred daily.

Symbiosis in lactic cultures. H. C. Olson, Oklahoma State University, Stillwater.

Pure cultures of *Streptococcus lactis* and citric acid fermenters were grown in combinations with other strains from the same and from different sources. Among the *S. lactis* strains some combinations of two strains were much more active in producing lactic acid than either of the parent strains. Likewise, combinations of other strains were much less active than either parent strain. It appeared that, in the former case, a symbiotic relationship existed, in that the cultures were mutually beneficial. In the latter case, it appeared that the cultures were antagonistic toward each other.

In some instances, combinations of *S. lactis* with citric acid fermenters yielded cultures that were more active than the parent cultures. In other instances, the addition of citric acid fermenters to *S. lactis* cultures lowered the activity.

Various combinations of commercial cultures, containing both the *S. lactis* and the citric acid fermenting types, were made and the acid-producing activities tested. Some combinations were more active than either parent culture, some were less active, and with some there was no change.

Study of the bacteriological and storage properties of cottage cheese. J. J. Sheuring, University of Georgia, Athens.

One hundred thirty-four samples of cottage cheese were observed for 2 yr. Many of the samples were secured from retail stores, while others were observed from the time they were manufactured and through the distribution processes to the consumer. All samples were analyzed for pH, percentages of fat, and total solids. Bacteriological tests included coliform, psychrophiles, yeasts, molds, and total bacteria.

Results indicated that most of the cottage cheese had a very high coliform count from the time it was manufactured until it reached the consumer. High percentages of the samples contained excessive numbers of yeasts, molds, and psychrophiles. Poor standardization of the cheese was indicated by the wide variation in percentages of fat in numerous samples.

Organoleptic tests were made according to the standards in the official cottage cheese score-card. The rapid deterioration of the samples from the manufacturer to purchaser indicates that much needs to be done to improve distribution methods.

Low-calcium media for control of bacteriophage in lactic cultures. H. C. Olson, Oklahoma State University, Stillwater.

Since bacteriophage does not proliferate in media of very low calcium content, such media have been applied to lactic cultures. A low-calcium milk designated PRM (phage-resistant medium) has proven successful for freeing cultures of bacteriophage infection. In our laboratories, various methods of treating milk were used to reduce the calcium to the point where the cultures grew well but the phage would not multiply. Certain phosphate salts were only partially successful. Treatment of milk with a synthetic resin base exchange system resulted in a low calcium product; however, this was not effective in preventing bacteriophage development.

Treatment of milk with 0.5% ammonium oxalate precipitated the calcium, and the supernatant liquid was successfully used in propagating phage-infected cultures. The addition of 0.5% corn steep liquor improved the rate of acid development in this medium. In either PRM or the oxalate-treated milk phage infected cultures could be freed of the infection after four or five transfers with a 1% inoculation rate.

A study of consumer handling practices affecting the quality of ice cream and mellorine. Carl Vanderzant and Randall Stelly, Texas Agricultural Experiment Station, College Station.

Information on this subject was obtained from 741 families in three cities in central Texas. Nearly all respondents indicated that the ice cream on purchase was in a desirable condition with respect to firmness. Approximately 10% of the families reported that some of the product normally was spilled over the package. The time interval between taking ice cream out of the store freezer and placing it in the home refrigeration unit was less than 5 min. for 13%; 5-10 min. for 25.1%, 11-20 min. for 41.2%, and more than 20 min. for

32.4% of the families interviewed. More than 81% of the families consumed all ice cream within a week from the day of purchase. Nearly all families indicated that the quality of the ice cream was good or very good when they consumed the first portion. However, 48% of the families noticed a change in quality after storage at home. The principal changes mentioned were softer and icy. Approximately 20% of the consumers interviewed did not like fruit ice cream. Main criticisms offered were lack of fruit and lack of true, fresh fruit flavor.

PRODUCTION SECTION

Evaluating dairy sires of three breeds at different levels of production. V. L. Baldwin and N. R. Thompson, Virginia Polytechnic Institute, Blacksburg.

Production records of daughters of sires have been compared with average herd production for comparable years. Daughter records were adjusted to a 305-day mature equivalent basis. Herd averages were from actual DHIA records during calendar years.

Performance of individual sires may be indicated for individual herds by graphing and comparing several levels of herd average groups and the average of daughters within them. Graphing of regression trends also will be helpful. Abnormal influences affecting herd average must be considered.

Evaluation of one Holstein sire showed 279 daughters with 417 records in 133 Virginia herds. Daughters' average was 439 lb. fat. Over-all herd average was 406 lb. fat. Regression of daughter average on herd average was 0.85. Nine other Holstein sires having three to 186 daughter records (total 603) averaged 497 lb. fat. Over-all herd average was 454 lb. fat. Daughter-herd regression of 0.88 was highly significant.

Six Guernsey sires had 15 to 83 daughter records (total 285) averaging 439 lb., with herd average 388 lb., and highly significant regression 0.67.

Two Jersey sires had 24 daughter records averaging 467 lb. fat, with herd average 423 lb. fat, and nonsignificant regression of −.06.

Regression coefficients varied widely among individual sires in all breeds.

A preliminary report on the use of tranquilizers in connection with electro-ejaculation of dairy bulls. W. N. Philpot, S. D. Musgrave, E. W. Jones, and W. E. Brock. Oklahoma Agricultural Experiment Station, Stillwater.

The purpose of this experiment was to gain information on several factors concerning the use of tranquilizers in connection with electro-ejaculation of dairy bulls.

A total of 13 bulls was utilized for varying lengths of time during the 11-mo. trial period. The death of three of the bulls utilized was associated with repeated use of one of the tranquilizers.

The tranquilizers were effective in reducing tetany of the rear quarters in a majority of the cases. The average volume and pH of the ejaculates collected by means of the electro-ejaculator were considerably higher than for those collected with the artificial vagina. The average initial motility and the average length of time which the samples remained motile were considerably reduced for those ejaculates collected by means of electro-ejaculation.

Influence of certain milk and egg yolk–citrate extenders on the motility of bovine spermatozoa stored at *5* and *−79°* C.* W. Grana and O. T. Stallcup, University of Arkansas, Fayetteville.

A comparative study of the motility of bovine spermatozoa extended in four extenders was carried out at a storage temperature of −79° C. Each 100 ml. of an egg yolk–citrate extender (I) contained 7 ml. of glycerol, 25 ml. of egg yolk, and 68 ml. of a 2.94% sodium citrate solution. The egg yolk–buffered solution (II), as described by Blackshaw, contained 7 ml. of glycerol, 25 ml. of egg yolk, and 68 ml. of a buffer solution (40% 0.1 *M* sodium citrate dihydride solution, 5% 0.1 *M* Na_2HPO_4 solution, 5% 0.1 *M* NaH_2PO_4 solution, and 1.25 g. arabinose). A third extender (III) was composed of 7% glycerol and 93% heated homogenized milk. The fourth extender (IV) used was a chemically treated milk composed of 7% glycerol, 91% homogenized milk, and 2% of a 1:100 dilution of a 70% thiovanic acid solution in a 2.94% sodium citrate dihydride solution.

The average motility of ten ejaculates subjected to treatments and stored for 15 days at −79° C. were 59, 57, 68, and 64%, respectively, for the four extenders.

When the glycerol level was increased from 7 to 10% at the expense of the buffer solution or milk, the average motility after 15 days of storage at 79° C. was 46, 32, 55, and 58%, respectively, for the four extenders.

The same four extenders previously used with three glycerol levels (0, 7, and 10%) were compared as to per cent motility after five days of storage at 5° C. It was found that the 7% glycerol level was superior to the other treatments. The heated, homogenized milk extender with 7% glycerol level was superior to the other extenders for storage at 5° C.

The role of cervicovaginal mucus in the preservation of bovine spermatozoa. W. E. Moss and Durward Olds, Kentucky Agricultural Experiment Station, Lexington.

Live sperm were recovered from the cervix of four cows 26-30 hr. after insemination

* Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

during estrus. When four other cows were inseminated during diestrus, the sperm remained viable only about 5-7 hr. In the laboratory, sperm lived about 5-7 hr. in either estrous or diestrous mucus under aerobic conditions at 37° C. However, anaerobically the sperm lived 10-15 hr. Use of a flow dialysis apparatus in an effort to simulate natural conditions for circulatory removal of waste products did not improve livability, although this may have been due to a loss of essential ions into the buffer medium. The acid hydrolysis of mucus did not appear to yield useful nutrients for sperm, as indicated by 119 oxygen uptake and livability studies.

Reconstituted buttermilk as an extender for frozen bovine semen storage. R. M. Myers, N. Pati, and L. C. Ulberg, North Carolina Agricultural Experiment Station, Raleigh.

A series of trials was conducted to determine the suitability of glycerolized, reconstituted dried buttermilk as an extender for frozen bull semen stored at −79° C. This extender, composed of 12 g. of dried buttermilk per 100 ml. of double-distilled water and with 9% glycerol added, was compared with a 2.9% sodium citrate–egg yolk extender containing 8% glycerol and with a heated, homogenized whole milk extender containing 10% glycerol. Semen from nine Holstein bulls in the N.C. Institutional Breeding Program stud was used and four replicates—a total of 72 ejaculates—were studied. A split ejaculate technique was used. The semen was extended at a rate of 20 million live cells per milliliter, cooled to 4.5° C. over a 4-hr. period, glycerolated, equilibrated for 18 hr., and frozen gradually in dry ice and alcohol. It was held at −79° C. for ten days, thawed at 0° C., stained differentially, and examined. The mean percentages of live sperm following this storage interval were: buttermilk 50.9, egg yolk–citrate 45.5, heated whole milk 45.1—a highly significant difference in favor of buttermilk. Under the conditions of these trials, second ejaculates were superior to first ejaculates in freezability. Semen from one of the nine bulls did not exhibit as high a percentage of recovery of live sperm when frozen in buttermilk as it did in the others. A subsequent series of trials has shown the buttermilk extender to be superior to either egg yolk–citrate or heated milk in approximately the same degree as the first series.

Optimum concentration of reconstituted buttermilk as an extender for bovine semen. N. Pati, St. Augustine's College, Raleigh, North Carolina.

A total of 100 split ejaculates from seven bulls was used in the semen dilution trials. Semen was extended with concentrations of reconstituted buttermilk ranging from 8 to 18%, to study the effect of the extenders on motility of sperm during ten-day storage at 40° F. The 12% concentration was found to be the approximate optimum for maintaining maximum livability of bovine sperm. A highly significant difference was observed in the motility of spermatozoa extended with reconstituted buttermilk (12%) in comparison with egg yolk–citrate over a ten-day storage period at 40° F. The reconstituted buttermilk (12%) was found to be superior to egg yolk–citrate in prolonging the livability of spermatozoa.

Effectiveness of streptomycin in controlling Vibrio fetus in stored semen. M. E. Wells, S. D. Musgrave, and E. W. Jones, Oklahoma Agricultural Experiment Station, Stillwater.

Four types of semen storage, designated as treatments, were used to study the effects of penicillin and dihydrostreptomycin sulfate on *Vibrio fetus* organisms purposely instilled into bovine semen. Treatment I, employing 500 units of penicillin and 500 μg. of streptomycin per milliliter, was stored by freezing at −79° C. Treatments II and III, employing 1,000 units of penicillin and 1,000 μg. of streptomycin per milliliter, were stored at 5° C. Treatments I and II were incubated for 1 hr. in a beaker of warm water (30-35° C.), then refrigerated for subsequent processing. Treatment III was refrigerated immediately after initial dilution. Treatment IV, employing 1,000 units of penicillin and 1,000 μg. of streptomycin per milliliter, was stored at room temperature. Control samples containing no antibiotics were prepared for all treatments.

Treatments I, II, and IV were completely effective in controlling *V. fetus*. Viable organisms were recovered from four (11.1%) of 36 treated samples of Treatment III. This indicated that the antibiotics were most effective when the diluter-antibiotic-bacterial mixtures were incubated for 1 hr. at 30–35° C. before subsequent processing. Viable organisms were recovered from 160 (96.3%) of the 166 control samples used in all treatments.

Resistance of Vibrio fetus to erythromycin lactobionate in semen stored at 5° C. M. E. Wells, S. D. Musgrave, and E. W. Jones, Oklahoma Agricultural Experiment Station, Stillwater.

Studies were conducted to test the effects of erythromycin lactobionate on bovine sperm and *Vibrio fetus*. Levels up to 5,000 μg. per milliliter produced no detrimental effect on visible sperm characteristics. No significant difference ($P < .01$) was found between levels from 500 μg. to 5,000 μg. per milliliter. *V. fetus* was instilled into two groups of diluted semen samples containing 500 and 2,000 μg. of erythromycin per milliliter. These groups were split into two subgroups. One subgroup of each level was refrigerated immediately after dilution. The second subgroup of each

level was incubated for 1 hr. in a beaker of warm water (30–35° C.), then refrigerated. Cultures were taken at 6, 24, and 72 hr. At the 500-μg. level, viable organisms were recovered four times from 15 treated samples held 1 hr., and seven times from 15 treated samples that received no holding time. At the 2,000-μg. level, the organism was recovered two times from 15 treated samples held for 1 hr. and four times from 15 treated samples that were refrigerated immediately. These results indicate that erythromycin lactobionate does not adequately control *V. fetus* under these conditions.

Differential effect of season on conception rate in Holstein and Jersey cattle. J. M. Hillin and I. W. Rupel, A & M College of Texas, College Station.

The breeding records of 642 first calf heifers and for 1,656 conceptions by 593 cows of the Holstein and Jersey breeds were studied to determine the influence of age of animal and season of the year on the number of services required for conception.

The Holstein heifers required an average of 2.34 and the Jerseys 2.09 services per conception. Holstein heifers, bred for the first time during the period June through October, required an average of 3.12 services per conception; whereas, Jersey heifers required 2.13 services during the same period. The services required per conception during the seven cooler months were 2.11 and 2.09, respectively, for the Holsteins and Jerseys.

For conception in cows with first services occurring in the five warmer months, an average of 2.36 services was required in Holsteins and 2.02 in Jerseys, as compared to 1.87 and 1.96, respectively, for the two breeds in the seven cooler months.

There were no significant differences with respect to age at first service for all heifers or for the age groups in Holstein cows. The Jersey cows of 8 yr. or older required a significantly higher number of services than other age groups within the breed.

Seasonal fluctuations in reproductive efficiency of dairy cows. H. A. Poston, R. M. Myers, and L. C. Ulberg, North Carolina Agricultural Experiment Station, Raleigh.

A 10-yr. summary of the breeding records from six herds of the North Carolina Institutional Breeding Program demonstrated that the per cent of cows bred which returned for second service within 60-90 days gradually increased from a minimum of 38% in January to a maximum of 56% in August. In general, these cows were bred at first estrus subsequent to 70 days postpartum. Further examination of 2,541 individual records revealed that the month of freshening had an effect on the subsequent calving interval. A maximum interval of 422 days was observed for cows freshening in May, after which there occurred a monthly decline to a minimum of 397 days for cows freshening in October. The records for each herd showed similar patterns.

A similar group of cows with 228 records, obtained with more detailed information over a 24-mo. period from one herd, demonstrated that the average interval from calving to subsequent successful mating (or to time of culling) ranged from a maximum of 184 days for April calvings to a minimum of 79 days for cows calving in June. However, there were only three observations for the month of June.

Fluctuations in interval from 70 days postpartum to first subsequent estrus closely approximated the pattern of variations in length of time from calving to subsequent successful mating or to culling. These data can be interpreted to mean that long calving intervals are partly due to failure of estrus. However, when the above records were analyzed for number of services required per successful mating, it was further demonstrated that cows with longer intervals from calving to successful mating also tended to require more inseminations before being diagnosed pregnant. This number of services increased from a minimum average of 1.7 for cows calving in December to 3.0 for cows calving in April.

Effect of certain environmental factors upon milk production in Georgia. O. T. Fosgate and H. K. Welch, University of Georgia, Athens.

Environmental, milk, and butterfat records were studied using DHIA records from 22 herds for the year of 1958. Records from seven herds of mixed breeds were used to evaluate the following variables: (1) management practices, (2) feeding practices, (3) maximum daily temperature, (4) season of calving, and (5) breed, upon the production of 4% F.C.M. Variables 1-3 were recorded on a herd basis and Variables 4-5 on an individual cow basis. Partial regressions and multiple coefficients of determination (R^2) were computed for Variables 1-3. Partial regression coefficients for feeding practices were significant for all breeds. However, R^2 values were only 3.5, 5.5, 6.0, and 2.8% for all breeds combined—Jerseys, Guernseys, and Holsteins, respectively. Winter and spring calving cows outproduced cows calving in the summer or fall.

Records from the 15 highest DHIA herds were used to study the effects of season of calving and breed upon the production of 4% FCM and butterfat (BF). Regressions due to season of calving on FCM and BF were obtained as follows: (a) fall −297.01 and −10.98; (b) winter 242.53 and 7.74; (c) spring 522.55 and 19.32; and (d) summer −468.07 and −16.07. Differences in production (FCM and BF) between the Holstein breed and the other two breeds were highly significant.

Effect of fiber content of the ration on the performance of dairy cows under hot and cool conditions. C. P. Breidenstein, J. E. Johnston, G. A. Hindery, and L. L. Rusoff, Louisiana Agricultural Experiment Station, Baton Rouge.

Twenty lactating cows (16 Holsteins and four Jerseys) were used in a replicated randomized-block design to study the effects of two rations, containing 22 and 32% crude fiber, respectively, at two temperature levels. The experiment was conducted over a period of 50 days. Normal outside temperatures during March and simulated hot summer conditions, with the temperature cycled daily from a minimum of 75° F. to a maximum of 95° F., were used. The four treatment combinations were: (I) 32% fiber and summer conditions, (II) 22% fiber and summer conditions, (III) 32% fiber and cool conditions, and (IV) 22% fiber and cool conditions. The basal low fiber ration consisted of an 18% protein concentrate, citrus pulp, and U.S. No. 1 alfalfa hay. The high fiber ration consisted of the basal ration with the addition of Solka-floc (purified wood pulp). All rations were fed according to the TDN requirements of the animals at 120% of Morrison's standards. The concentrate portion of the ration was fed at the rate of 1 to 4 FCM.

The average daily production of FCM on the four treatments was 29.4 lb. for treatment (I); 30.2 lb. for (II); 30.6 lb. for (III); and 32.5 lb. for (IV). The differences in milk production among treatments were significant at the 1% level of probability.

Complete chemical and statistical analyses of the data will be presented.

Effects of protein content of the ration on the performance of dairy cows in warm weather. R. E. Leighton and I. W. Rupel, Texas Agricultural Experiment Station, College Station.

In a further study of rations best suited for producing dairy cows in hot weather, the effects of adequate versus high levels of protein were compared. A high energy (low fiber) ration designed to meet the protein requirements of the cows was compared with a similar ration which supplied 50% in excess of the calculated protein requirements. Estimated net energy supplied by the two rations was equal.

There were no statistically significant differences in the production, body temperatures, pulse rates, and respiration rates of the cows when fed the two rations, but the observed trends favored the high protein diet. The average daily milk production per cow, body temperature, respirations per minute, and pulse beats per minute were 0.15 lb. higher, 0.07° F. lower, 0.71 slower, and 0.41 slower, respectively, when the cows were fed the high protein ration.

Effect of varying quantities of feed on milk production in DHIA herds. William S. Griffith and K. E. Loope, Virginia Polytechnic Institute, Blacksburg.

Production and feed records for the year 1958 on 47,850 cows in 970 Virginia DHIA herds were grouped according to levels of butterfat production. Standard market values were assigned to hay, silage, and grain. Pasture was not measured. Most of the difference in average level of production per cow can be explained by differences in the quantity of feed provided. Maximum returns over feed cost were realized at the higher levels of production. Return over feed cost alone does not accurately depict the relationships in net income. Great improvements in production per cow in the South could be realized by strengthening feeding programs first.

Relationship of lignin to other chemical constituents in Sudan and millet forages. A. S. Achacoso, C. L. Mondart, Jr., F. L. Bonner, and L. L. Rusoff, Louisiana Agricultural Experiment Station, Baton Rouge.

The lignin content and other chemical constituents of two summer pasture grasses, as well as the relationship among lignin, crude protein, crude fiber, and dry matter, as affected by stage of maturity and rates of nitrogen fertilization, were studied. Sudan and millet forages, fertilized with 0, 30, 60, 120, and 240 lb. of nitrogen per acre, in two applications, were cut at ten-day intervals. First cuttings on all treatments were made when the plants were 30-35 in. high, and were cut back to a 6-in. stubble.

Results on both forages showed that lignin progressively increased with plant maturity, with values ranging from 3.23 to 6.72% on the first and fifth cuttings, respectively. The percentages of crude protein decreased significantly from 13.1 to 5.9% with a relatively small decline between the first two cuttings; whereas, crude fiber increased significantly, with a decline at the fifth cutting, which was apparently due to seed formation, particularly in the millets.

No significant increases in lignin up to 120 lb. of nitrogen application per acre were obtained. Crude protein increased significantly from 7.4 to 13.5%, whereas crude fiber decreased from 32.0 to 20.1% at the 0- and 120-lb. levels, respectively. However, there were no significant differences in the crude fiber content at the 30-, 60-, and 120-lb. levels. Dry matter, nitrogen-free extract, and ash also increased.

Highly significant negative correlation coefficients were obtained between lignin and crude protein, crude protein and crude fiber, and crude protein and dry matter. Positive correlations were obtained between lignin, crude fiber, and dry matter at the 1% level of probability.

Effect of protein supplementation on consumption and digestibility of sorghum silage.

C. B. Browning, J. W. Lusk, and J. T. Miles, Mississippi State University, State College.

A 4×4 Latin-square design, using eight heifers (average body weight, 825 lb.), was used to compare the following crops ensiled in the soft dough: Tracy sorghum, oats (wilted), corn, and Tracy sorghum + 2 lb. of soybean oil per day. Experimental periods were 14 days in length. Total feces collections were made the last seven days of each period. The voluntary silage dry matter consumption (lb/100 lb. body weight) for the four treatments, respectively, was 0.89, 1.26, 1.23, and 1.22. The digestibilities of the silages were as follows: dry matter 54.7, 60.8, 58.1, and 55.7; protein 19.3, 60.9, 46.0, and 13.4; and energy 56.8, 60.2, 60.2, and 60.6. A second trial, using a 3×3 extra-period Latin-square design and six heifers, was used to compare the same sorghum alone and supplemented with either 2 lb. of SOM or 160 g. of 42% urea per day. The consumption and digestibility of the silage in the three rations were: DM consumption per 100 lb. of body weight 0.91, 1.38, and 1.11; DM digestion 46.5, 52.8, and 50.2; protein digestion 19.6, 18.5, and 21.3; and energy digestion 48.0, 57.0, and 52.5.

Factors affecting heifer performance on silage rations. M. E. McCullough and W. E. Neville, Jr., Georgia Experiment Station, Experiment.

Three silages were individually fed to dairy heifers averaging about 400 lb. in body weight, to study certain factors relative to animal performance. Silage 1 was a pre-bloom oat silage without preservative, Silage 2, an early-bloom alfalfa preserved with 200 lb. ground snap corn per ton, and Silage 3, the same alfalfa preserved with 80 lb. molasses per ton. Ten heifers were used with Silage 1 and 12 heifers with each of Silages 2 and 3. Grain was fed to six heifers on Silages 2 and 3 to provide 50% of calculated maintenance requirement. Average daily dry matter intakes and daily gains were: Silage 1, 7.41 and 0.93 lb.; Silage 2, 11.31 and 1.81 lb.; Silage 3, 10.50 and 1.61 lb. Correlations between A.D.G. and intake of forage were .711, .470, and −.265, respectively. Correlations of .932, .740, and .689 were obtained betwen body weight and forage intake. Where the data were examined by multiple regression, intake of forage accounted for 59, 50, and 6% of the difference in average daily gain. Size of animal as measured by body weight was important only for Silage 1. Grain feeding accounted for 43% of the variation in gain with Silage 3 and for 20% with Silage 2. Since the same heifers were used with Silages 2 and 3, it was possible to calculate the repeatability of intake for these heifers. When the data were corrected for grain and silage effects, the repeatability was .860.

Preservation and feeding value of Starr millet and Sundan grass silage for dairy cows. L. R. Sisk, M. E. McCullough, and O. E. Sell, Georgia Experiment Station, Experiment.

Twenty-eight-day feeding trials were conducted, using five or six cows, to evaluate the silages listed as follows: Silage No. 1, boot-stage Sudan grass (1956); No. 2, boot-stage Sudan grass (1957); Nos. 3 and 4, early heading–stage Sudan grass plus cowpeas in early-bloom stage (1958); No. 5, early heading–stage Starr millet (1958); Nos. 6 and 7, early heading–stage Starr millet (1959). Silages 1 and 2 were made without a preservative. Silages 3 through 7 were ensiled with either 100 or 200 lb. of ground snap corn per ton of green forage. Average daily intakes per cow were 141, 119, 115, 101, 109, and 98 lb. for Silages 1 through 6, respectively (Silage No. 7 data to be completed). The 28-day changes in FCM were −15, +5, +5, +12, +4, and −7%, respectively. There was no significant difference in dry matter preservation due to the addition of 200 lb. of snap corn instead of 100 lb.

Intake and digestibility of Coastal Bermuda grass and Bahia grass as affected by management. George E. Hawkins and G. H. Rollins, Louisiana State University, Baton Rouge.

Intake and digestibility of Coastal Bermuda grass and Pensacola Bahia grass under continuous and 1-wk. rotational grazing were determined by chromogen–chromium oxide ratio technique at 3-wk. intervals from June 30 to September 16, 1958. Twelve experimental paddocks (six Coastal Bermuda grass and six Bahia grass) supplied the grazing for this test. Three paddocks of each species were grazed continuously and three rotationally by one lactating cow per paddock during each test period.

Forage dry matter intakes were not affected significantly by species or management. Intakes of forage dry matter in pounds per 100 lb. of body weight were: CC (Coastal Bermuda grass, grazed continuously), 2.27; CR (Coastal Bermuda grass, grazed rotationally), 2.53; BC (Bahia grass, grazed continuously), 2.36; and BR (Bahia grass, grazed rotationally), 2.13. The L.S.D., $P = 0.05$ was 0.47. Mean forage dry matter intakes in pounds per 100 lb. body weight by periods were: I, 2.52; II, 2.49; III, 2.23; and IV, 2.06. Coefficients of digestibility of forage dry matter by treatments were: CC, 59.1; CR, 59.5; BC, 58.6; and BR, 60.3. The L.S.D., $P = 0.05$ was 1.35. There was practically no difference in the coefficients of digestibility of forage dry matter during Periods I and II. However, the digestibility of the forages decreased at a rapid rate during Periods III and IV. The coefficients of digestibility of dry matter by periods were: I, 61.9; II, 62.3; III, 58.2; and IV, 55.2. Seventy-three per cent of the variance in persistency of milk production associated with management of pastures was

related to differences in digestibility of forage dry matter.

Nutritive value of Pearl millet silage preserved with various antibiotics. J. M. WING AND C. J. WILCOX, University of Florida, Gainesville.

Five other antibiotic-treated silages were compared to one containing zinc bacitracin, an accepted antibiotic for silage preservation, and to fresh forage by means of digestion trials involving eight heifers each. Run-off was analyzed for relative numbers of proteolytic and other bacteria. In addition, each silage was fed to four lactating cows for three days, and the milk examined for antibiotic activity. All antibiotics (5 g/ton) were mixed into the forage by self-unloading wagons. Silos (capacity six tons) were of polyvinyl-treated canvas. An air-tight seal was accomplished by means of a combination liner and cover of 8-mil polyvinyl film. The pH, % TDN and DCP (dry), and daily consumption in lb/1,000 lb. body weight were: green forage, 5.0, 59, 5.1, 102; zinc bacitracin: 5.0, 61, 6.3, 94; Aureomycin: 5.2, 62, 7.1, 65; Oleandomycin: 5.0, 59, 7.4, 102; streptomycin: 4.9, 66, 7.2, 89; Terramycin: 4.0, 67, 7.8, 72; and penicillin: 4.5, 70, 8.6, 101. Light proteolytic activity was observed in Aureomycin silage only. Other bacterial activity appeared light with Oleandomycin and moderate in all others. Oleandomycin only was suspected of carrying through into the milk of one cow.

Effects of feeding aureomycin to lactating dairy cows. LOUIS J. BOYD, H. D. BAXTER, J. B. MCLAREN, AND J. R. NICHOLS, Tennessee Agricultural Experiment Station, Knoxville.

Sixty-two pairs of cows (33 pairs of Jerseys and 29 pairs of Holsteins) in four herds were used to determine the response from adding aureomycin to the dairy ration. After the antibiotic was introduced gradually into the ration of the treated cows, the experiment was run for 12 wk. Seventen pairs in one herd were continued for 17 wk. and eight pairs in another herd for 25 wk. Aureomycin was fed at the rate of 10 mg. per 100 lb. average body weight. All cows of the same breed in each herd were fed the same amount of aureomycin daily.

The average differences in milk production between the two groups of cows were not significant. The average daily production of 4% FCM during the 12-wk. period was 32.7 lb. for the controls and 33.0 lb. for the supplemented group. The aureomycin-fed cows started at a slightly higher daily production. Both groups declined at approximately the same rate throughout the study. Regardless of the length of the treatment period, differences between the two groups of cows in body weight changes or in the resistance to mastitis, foot rot, or other bacterial disturbances were not significant. No harmful effects were apparent from feeding aureomycin for the periods indicated.

Alfalfa grazing and its contribution to pasture mixtures for dairy cows. W. R. MURLEY, W. B. GILBERT, AND H. D. GROSS, North Carolina Agricultural Experiment Station, Raleigh, and M. L. SELF, Piedmont Research Station, Salisbury, North Carolina.

Twenty-four acres were grazed with 24 lactating cows and 12 dairy heifers, to measure the productivity of various pasture mixtures and systems of grazing. The following species of forages, replicated three times, were seeded in the fall, 1958, and grazed during the summer, 1959: 1. Ladino clover–orchard grass (2 acres); 2. Ladino clover–orchard grass (1 acre) and alfalfa (1 acre); 3. Ladino clover–orchard grass (1 acre) and alfalfa–orchard grass (1 acre); and 4. Orchard grass (two acres). Treatments 1 and 4 were grazed continuously, whereas 2 and 3 were grazed in a system whereby the alfalfa and the alfalfa–orchard grass, respectively, were strip-grazed for seven-day periods and then the Ladino clover–orchard grass plots were grazed continuously until the alfalfa had recovered. The first growth in the spring was removed for silage. The treatments provided 117, 116, 114, and 84 cow days grazing per acre, respectively, and yielded 1,594, 1,698, 1,404, and 970 lb. calculated TDN per acre and supported 3,366, 3,152, 3,046, and 2,180 lb. of FCM per acre, respectively. The botanical composition of the forages withstood the treatment during the year and promises one or more additional years of experimental grazing.

Lactation performance of dairy cows grazing certain annual and perennial grasses in Alabama. I. Coastal Bermuda grass, Pensacola Bahai grass and Dallis grass. G. H. ROLLINS, C. S. HOVELAND, W. R. LANGFORD, R. D. HICKS, AND R. A. BURDETT, Alabama Agricultural Experiment Station, Auburn.

Three experiments were conducted: I, June 10–October 18, 1956; II, May 8–October 22, 1957; III, May 14–August 26, 1958. Grain to F.C.M. ratios were 1:4 initially and were adjusted for all cows as production declined. To use all available forage, animals were added as needed according to the put-and-take system. Relative forage yields were determined: (1) as calculated TDN yields per acre based on animal performance, and (2) by cage-difference technique.

With continuous grazing, F.C.M. production was not affected by species or by irrigation when cows with a starting mean daliy F.C.M. production of 29.1 lb., Experiment I, and 36.7 lb., Experiment II, grazed Coastal Bermuda, Pensacola Bahia, and Dallis grass. Mean daily F.C.M. for the final week of Experiments I and II were 18.4 and 14.6 lb., respectively.

In Experiment III, all paddocks were irrigated. Daily F.C.M. production of cows fed alfalfa hay (mean 22.4% C.P., dry basis) in dry lot was greater ($P<.01$) than that of cows grazing Bahia or Coastal Bermuda ro-

tationally, or grazing Coastal continuously, and greater ($P<.05$) than that for Bahia grazed continuously. Mean F.C.M. from Bahia grazed rotationally and continuously was higher ($P<.05$) than that from Coastal Bermuda grazed continuously.

Lactation performance of dairy cows grazing certain annual and perennial grasses in Alabama. II. Tall fescue, oats, and wheat. G. H. ROLLINS, C. S. HOVELAND, W. R. LANGFORD, AND R. A. BURDETT, Alabama Agricultural Experiment Station, Auburn.

A series of experiments was conducted at Auburn, Alabama, to compare the production performance of lactating cows grazing certain cool season grasses. Grain was fed at a grain to F.C.M. ratio of 1:4. Chopped alfalfa hay (mean 23% C.P., dry basis) was used as a roughage supplement, or control, in some of the experiments.

During Experiment I (March 21–May 8, 1958), no differences were found in F.C.M. production of groups of cows grazing: (1) tall fescue, (2) oats, (3) fescue + hay, and (4) oats + hay. Mean daily F.C.M. for all groups of cows during the experiment was 34.7 lb., 101.1% of that for the standardization period.

In Experiment II (October 3–November 13, 1958) mean daily F.C.M. for fescue (46.0 lb.) was not significantly different from that on alfalfa hay fed in dry lot.

Mean F.C.M. production on wheat (43.1 lb.) in Experiment III, (November 14–December 19, 1958) was higher ($P>.05$) than on alfalfa hay in dry lot (39.1 lb.).

Effect of different rations fed in the dry period upon the next lactation. E. W. SWANSON AND S. A. HINTON, University of Tennessee, Knoxville.

Eighty cows paired on the basis of previous lactations were compared in three trials in a 3-yr. period. During the dry period, all cows were fed hay and silage ad libitum under dry-lot conditions in outside bunks. One of each pair was also brought into the milking room and fed 8 lb. grain daily for the last 6 wk. of the dry period. The cows of each pair were fed the same allowance of grain for 24 wk. after calving and then were fed according to milk production. Body weights and milk samples were taken weekly. Dry periods averaged 65 days for the grain fed and 72 days for the roughage fed cows. Grain was fed an average of 38.4 days (307 lb. grain) in the dry period. The grain fed cows gained an average of 45 lb. in the last 6 wk. of gestation, compared to 22 lb. gained by those fed only roughage. The grain fed cows started lactation at a higher level than the roughage fed cows each year. In the first 30 wk. of lactation those fed grain in the dry period produced 7,860 lb. FCM, which was 4% more than the 7,531 lb. FCM from the roughage fed cows. In the preceding lactation, the groups had averaged the same FCM yields; dry period grain feeding caused a slight increase, equal to about 1 lb. FCM for 1 lb. dry period grain fed.

Factors for extending incomplete lactations of Jersey and Sindhi-Jersey cows. J. L. FLETCHER, Iberia Livestock Experiment Station, Jeanerette, Louisiana.

Dairy cattle breeding research frequently is handicapped by inadequate numbers. In many cases, lactations are interrupted by events which do not prejudice the record prior to the interruption. These part-records may be salvaged by use of satisfactory extension factors. Studies of the lactation records of Jersey and Sindhi-Jersey cows at the Iberia Livestock Station have provided evidence that the pattern of production departs from the classical lactation curve. It has further been noted that season of calving appears to alter the production curve.

The present report concerns a study of 264 Jersey and Sindhi-Jersey records made at this station between 1948 and 1956. In a preliminary effort, extension factors were derived from these records for each of the breed groups without consideration of season of calving. These factors showed rather good agreement with the Jersey extension factors reported by the Dairy Cattle Breeding Committee of the American Dairy Science Association, except for part-lactation of 90 days or less. For these short intervals, a somewhat smaller factor was satisfactory for extending the Jersey and Sindhi-Jersey records in this herd. This indicates less persistency of lactation in these cows, with this being somewhat more evident for the Sindhi-Jersey group.

In a more detailed study, records of each breed group were sorted by season of calving. Four seasons were used, beginning with the month of January. It was found that the major differences were again in the early part of the lactation. The smaller factors required for winter and spring lactations as compared to fall lactation indicate the need for considering adjustment for season of calving in extension factors applied to part lactation made in this herd.

Effect of fertilization on the crude fiber content and crude fiber-protein relationship of Coastal Bermuda grass. W. J. MILLER, J. D. DONKER, AND M. STELLY, University of Georgia, Athens.

Coastal Bermuda grass was grown on 192 plots consisting of three replications of a $4\times4\times4$ rates of N, P, and K factorial design. The levels of fertilization were: 0, 100, 200, and 400 lb. of N per acre and 0, 50, 100, and 200 lb. of P_2O_5 and K_2O each per acre. Samples of forage from the three replications were

composited from each of three clipping dates, making a total of 192 samples which were analyzed for crude fiber. All 576 samples were analyzed for N and the data for the three replications composited. The variations in P and K fertilization had no effect on the crude fiber or the Kjeldahl N content of the forage. With an increase in N fertilization, the N content of the forage increased, but the crude fiber was not affected. The N and crude fiber per cents on a dry matter basis, with 0, 100, 200, and 400 lb. of N fertilization, were, respectively: 1.6, 26.4; 1.8, 26.7; 2.1, 26.6; and 2.3 and 26.3. On the within-clippings basis, the correlation between crude fiber and N content of the forage was −.06.

Nitrate poisoning of dairy cattle grazing oat forage. O. T. Stallcup, R. Robberson, O. H. Horton, and R. L. Thurman. Arkansas Agricultural Experiment Station, Fayetteville.

Slight evidences of nitrate poisoning were observed in dairy cattle of the Arkansas Agricultural Experiment Station herd while they were grazing oat forage from land having a heavy application of chicken litter. Before being seeded to oats, the area was used as a range for pullets. Although scattered, the litter was concentrated where the range shelters were located. The oats received 300 lb. of 5-10-5 at planting time. No nitrogen was applied in the spring. From spots heavily coated with chicken litter, the oats contained 3.41% total nitrogen and 0.67% nitrate nitrogen on a dry matter basis. Plants from other areas of the same field contained 2.8% nitrogen and only 0.11% nitrate nitrogen. Forage from these areas was eaten first by the cows in preference to forage from the areas on which chicken litter was scattered.

Tests were made on a plot of fall seeded oats of the Arkwin variety during the spring of 1958. At planting time in the fall, 300 lb. of 5-10-5 fertilizer were applied per acre. On March 26, 1958, an application of 32 lb. of nitrogen in the form of ammonium nitrate was made per acre under conditions of low soil moisture. Samples of forage were collected and analyzed at weekly intervals until the grain was ready for harvest. The plant tissue was analyzed for total nitrogen, water-soluble nitrogen, and nitrate nitrogen. The total nitrogen in the forage reached the peak (3.9%, dry matter basis) within ten days following the application of nitrate fertilizer, then declined steadily. The water-soluble nitrogen in the forage, which includes ammonia nitrogen, nitrate nitrogen, and other nonprotein nitrogen, remained at a high level for 3 wk. following nitrate fertilization, then declined. The nitrate fraction reached 0.7% (dry matter basis) on April 18, 23 days following fertilization, then declined rapidly.

Effects of potassium nitrite on litter size and growth rate of rats. L. J. Boyd and S. Lane Parkinson, University of Tennessee, Knoxville.

Thirty-two mature female rats, divided into four groups, were injected intramuscularly and later fed KNO_2 daily to determine its effect on litter size, birth weight, and growth rate of their offspring. The highest level of KNO_2 when injected was 0.01125 g. daily, which was equivalent to 0.075% KNO_2 in the ration (based on 15 g. feed per rat daily). In Phase II, the levels of KNO_2 used previously were doubled, added to the ration, and fed daily.

Treatment began 1 wk. before breeding and continued until the offspring were 21 days old. All females were on treatment for at least 50 days. The litters were standardized to seven on the fifth day of age.

In Phase I, the controls averaged 10.3 rats per litter weighing 6.0 g. each, whereas the rats receiving the middle level of KNO_2 averaged 11.3 rats weighing 6.0 g. each. In Phase II, the rats receiving the highest level had the largest litters. The offspring from the controls made the greatest gains and the offspring from rats receiving the middle and highest levels of KNO_2 made the least gains in Phases I and II, respectively. None of the differences between groups was significant, regardless of the method used in administering KNO_2.

Some effects of tranquilizers in dairy cattle. J. M. Buckalew, Mississippi State University, State College.

Perphenazine and Chloropromazine, two of the commercially available tranquilizing drugs, have been studied with respect to their ability to combat certain stress conditions normally encountered in dairy cattle management. Intramuscular dosage at the rate of 1 mg. tranquilizer per 10 lb. body weight was used in an attempt to more easily acclimate first-calf heifers to the milking routine. After treatment, heifers displayed little nervousness and little or no inclination to kick, a sharp contrast to their behavior prior to tranquilization. A single dose of tranquilizer administered to cows just prior to their separation from their newborn calves at least temporarily prevented the disturbance in the herd commonly caused by these cows.

The following general effects have been observed: 1. At the dosage used, a calming effect was produced, without accompanying anorexia or loss of alertness. 2. It required from 1 to 2 hr. for the tranquilizer to take effect following intramuscular dosage, the time required varying among animals. 3. The duration of tranquilization varied, but appeared to be no more than 24 hr. in any animal. 4. Milk let-down appeared to be normal in all tranquilized animals.

Comparison of different levels of protein in a milk replacer for young dairy calves. L. J.

BUSH AND J. D. SCHUH, Oklahoma Agricultural Experiment Station, Stillwater.

Thirty Ayrshire bull calves were used in an experiment designed to compare semisynthetic milk replacers having different levels of protein. Dried skimmilk, animal tallow, lecithin, casein, lactose, and cerelose were used to formulate the replacers which were constituted to contain 13% total solids. The protein content of the three diets was calculated to be 15, 20, and 25%, respectively, on a dry matter basis. The total energy content of the three diets was the same, approximating that of whole milk containing 2.5% fat. The calves were fed the replacers at the rate of 10% of initial body weight, starting at three days of age and continuing throughout the 6-wk. trial. Vitamin and mineral supplements were added to the replacer once each day at the time of feeding. Observations on the incidence and severity of diarrhea were made twice daily and body weights were recorded weekly. Growth of the calves was variable within each of the three groups, yet greater on the average for the group receiving the higher level of protein. The incidence of diarrhea was similar for all three groups of calves.

Role of pregastric esterase in the hydrolysis of milk fat in the abomasum of young calves. H. A. RAMSEY AND J. W. YOUNG, North Carolina State College, Raleigh.

The role of pregastric esterase in the abomasal hydrolysis of milk fat was studied in two milk-fed, rumen-fistulated calves at 3 mo. of age. Raw whole milk, containing approximately 3.5% fat, either was fed from a nipple pail (oral feeding) or was placed directly into the abomasum via a rubber tube (abomasal feeding). The latter system was used to bypass the oral cavity, the site of pregastric esterase secretion. At 1, 2, and 3 hr. after feeding, the contents of the abomasum were sampled and subsequently analyzed for total free fatty acids (FFA). In addition, the FFA from the oral-fed samples were separated by column chromatography into three fractions: butyric, valeric, and higher fatty acids. The mean concentrations of total FFA (μM/5 g. abomasal contents) were as follows for the oral and abomasal systems of feeding, respectively: 1 hr., 86, 19; 2 hr., 101, 20; and 3 hr., 120, 30. For the oral-fed samples, the mean percentages of butyric, valeric, and higher fatty acids were as follows, respectively: 1 hr., 37, 8, 55; 2 hr., 22, 4, 74; and 3 hr., 10, 3, 87. Thus, pregastric esterase appears to effect considerable hydrolysis of milk fat in the abomasum, although the source of the valeric acid is obscure. The mean pH values of the samples for the oral and abomasal methods of feeding, respectively, were as follows: 1 hr., 4.87, 4.64; 2 hr., 4.01, 4.04; and 3 hr., 3.36, 3.37. Changes in the level of blood sugar after ingestion of milk were similar for both systems of feeding.

Central machine processing of DHIA records in the Southeast, its advantages and limitations. M. E. SENGER, North Carolina State College, Raleigh.

Methods and procedures have been developed whereby standard DHIA and owner-sampler production and feed records are processed on a current monthly basis by electronic computing machines at a central location. In this system the DHIA supervisor becomes a field reporter of production and feed data rather than spending considerable time with tedious hand calculations.

Production and feed data are mailed to the Dairy Records Processing Center, North Carolina State College, by the DHIA supervisor the same day the test is completed. At the Center the reports are audited, data transferred to punch cards, calculation made, results tabulated, and the monthly herd report mailed direct to the herd owner. This requires an average of 11 to 12 days from date of test until the herd owner receives his calculated report. There are numerous advantages to this system: (1) More complete and accurate DHIA records are provided. (2) Monthly and yearly herd data and test day, test period, and lactation-to-date data for each cow are given in printed form on a few pages. (3) Accumulating herd and individual cow lactation data are current and up-to-date with each monthly report. (4) More information, especially on feeding and labor, is given. (5) All 305-day and complete lactation records are calculated and reported automatically. (6) The DHIA supervisor's job is becoming more attractive and should reduce excess turnover in supervisors. (7) Supervisor has more time to provide information required in DHIA and to help dairymen use their records more adequately. (8) Puts DHIA production and feed data in a more usable form for use in teaching and research.

The limitations to this system consist mainly in the improper reporting of data, inaccurate evaluation of feeding, value of forages, delays in monthly reports getting back to the farm, and additional cost. The acceptance of this system by many DHIA members in a relatively short period of time is a good indication that its advantages strongly predominate over these limitations.

Daily observations on the summer production of fat and solids-not-fat by Jersey cows. J. C. JOHNSON, JR. AND R. L. GIVENS, Georgia Coastal Plain Experiment Station, Fifton.

No abstract.

REPORT OF SPECIAL AMERICAN DAIRY SCIENCE ASSOCIATION COMMITTEE ON PUBLICATION POLICY

This special committee met in Miami Beach, Florida, on October 7, 1959, with all members present. President A. C. Fay made all arrangements and joined the committee for the entire day's deliberation.

The charge to the committee dealt with the subject of Future Publication Policies of the Journal. This question grew out of increasing dissatisfaction among some of our membership who feel that our Journal, in many respects, is too technical even for those who have a strong interest in the field. On the other hand, some dairy scientists feel that our Journal is becoming too applied and practical, and that a further trend in this regard might jeopardize its status as a scientific Journal.

The committee decided that in order to see this problem intelligently we should first analyze the composition of our present membership and attempt to decide who should be encouraged to seek membership in our Association. When this is established we can then decide what publication policy can best serve the members and the industry.

After considerable discussion the committee agreed that the following groups should benefit from membership in the American Dairy Science Association:

a. University teachers and research workers in all phases of the dairy industry, as well as some allied industries, such as veterinary science, animal husbandry, and nutrition.
b. University and USDA extension workers.
c. USDA researchers.
d. Commercial enterprises.
    1. Dairy manufacturing and processing
    2. Dairy equipment and supply
    3. Chemical manufacturers of cleaners, baby foods, etc.
    4. Breed associations
    5. Artificial breeding associations
    6. Dairy and food researchers
    7. Dairy herd improvement associations
    8. County agents
    9. Farm managers
    10. Milk producer associations
    11. Allied food industries (research and technical workers)
e. Service or regulatory work.
    1. Federal, state, or city health personnel
    2. National Dairy Council
    3. Trade associations
    4. State departments of agriculture
    5. Milk marketing administrators and research workers.
f. Students.

Since the great majority of university teaching, research, and extension workers are members of the Association, our further growth must come from the other groups. It was estimated that our present membership is composed of about 50% university and 50% non-university people. The committee agreed that A.D.S.A. can and should grow and, in order to be of the maximum service to the Dairy Industry, our publication policy should encompass both the basic and applied aspects of Dairy Science. It was agreed that the Journal is now serving the needs of our basic scientists quite adequately, but that the people interested in the application of their research are in most cases unable to interpret the practical significance of the work. There also appears to be a need for publishing more applied research which has been conducted, and reporting it in an acceptable technical way.

In order to broaden our publication approach to make our Journal more acceptable to the present membership and more attractive to potential members, the committee considered the following alternatives:

1. Include a practical résumé and interpretation of all scientific articles following the Table of Contents in each issue of the Journal.
2. Publish the present Journal with two quarterly digests, one for manufacturing and the other for production.
3. Publish the present Journal with scientific papers plus Association notes only and then publish a monthly popular Journal containing both production and manufacturing papers and call it the JOURNAL OF APPLIED DAIRY SCIENCE.
4. Publish our present Journal containing a practical résumé and interpretation of scientific articles, except that two Journals would be published each month, one for manufacturing and one for production.
5. Same as 4, except following an alternate month publication; that is, the manufacturing issue one month and the production issue the next month.
6. Leave publication as it is.

Subject to the approval of the Board, it is recommended:

1. That this special committee continue its work and explore the feasibility, costs, and organization changes which would be required in the publication of two journals:
    a. JOURNAL OF DAIRY SCIENCE, to include both production and manufacturing papers of a basic scientific character.

b. JOURNAL OF APPLIED DAIRY SCIENCE, to include applied papers, with a digest of papers from the JOURNAL OF DAIRY SCIENCE. This Journal would also carry most of the advertising, popular notes, etc.

2. That this committee report its findings to the Board by June, 1961.
3. That, in the interim, the Journal Management Committee consider the immediate insertion of a section in the Journal giving a practical résumé and interpretation of the scientific papers in each issue of the Journal.[1]

[1] This action was recommended by the Journal Management Committee and approved by a majority vote of the Board of Directors. It was made effective January 1, 1960.—Editor-In-Chief.

At the suggestion of this special committee, President A. C. Fay has submitted recommendation 3 to the Journal Management Committee for its immediate action. A report from the Journal Management Committee to the Board will be made through the President at an early date.

Respectfully submitted,

Special Committee on Publication Policy

BURDET HEINEMAN
J. T. REID
W. M. ROBERTS
E. O. HERREID
H. F. JUDKINS
D. V. JOSEPHSON, Chairman

BOOK REVIEW

MACHINE MILKING. Published by the Ministry of Agriculture Fisheries and Food. Whitehall Place, London S.W. 1. Bulletin 177. 1959.

This bulletin contains 195 pages with 50 line drawings and 67 photographs. It was prepared under the general supervision of Dr. A. T. R. Mattick, with the help of the staff of the National Institute for Research in Dairying at Reading.

This bulletin is unique in that it combines the theory and practice of the milking process, starting with the anatomy and structure of the udder and the physiology of lactation. Mastitis is emphasized as a major problem in machine-milking.

The problems of maintenance, design, and construction of milk machines are considered in a very practical but detailed way. The construction, operation, and organization of milking parlors are illustrated to provide a sound basis for their construction.

The care, cleaning, and sterilizing of all milking equipment are described in detail. The atomic structure of the rubber molecule is shown and explanations given for the care needed to preserve and prolong the life of rubber inflations.

This bulletin is well written and very informative. The cost is 12s.6d., net.

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